USE OF VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI FOR ESTABLISHMENT OF EFFECTIVELY NODULATED LEGUMES ON MODERATELY WEATHERED OXISOL SUBJECTED TO SIMULATED EROSION

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ABSTRACT

The objectives of this study were to determine the influence of simulated erosion on the population and activity of vesicular-arbuscular mycorrhizal (VAM) fungi, and to define the levels of chemical inputs necessary for successful establishment of effectively nodulated and mycorrhizal cowpea (Vigna unguiculata) and leucaena (Leucaena leucocephala) in a soil subjected to simulated erosion.

Erosion was simulated by removing the top 30 cm of the Wahiawa soil (Tropeptic Eutrustox). Removal of top soil resulted in a significant decrease in the population and activity of VAM fungi. When the infectivity of soil was increased by inoculation with different strains of VAM fungus, there was no improvement in plant growth, indicating, that nutrients were, perhaps, limiting. Experiments were then conducted to determine the influence of P, lime, organic residue, Mo and inorganic N on mycorrhizal activity and plant growth.

The growth of mycorrhizal cowpea and leucaena was significantly improved when the eroded and uneroded soils were amended with phosphorus. The results showed that the level of P is very critical for the symbiosis between VAM fungi and hosts. There appear to be threshold, optimum and inhibitory levels of soil solution P for mycorrhizal activity. The optimum soil solution P level for mycorrhizal activity was found to be 0.026 mg/l. At this P level the

difference in plant growth that existed between the eroded and uneroded soils in the absence of added P disappeared. Liming the eroded soil to pH 6.0 was beneficial to mycorrhizal cowpea and leucaena. Amendment of the soil samples with organic residue and Mo was not beneficial to the test legumes whereas the application of inorganic N at the rate of 25 ppm increased the growth of mycorrhizal cowpea and leucaena in the eroded soil. Nodule dry weight and shoot N status of plants were also increased significantly by adding 25 ppm N to the soil. Maximum nodule dry weight was observed at 50 ppm N.

The nutrients (P, lime and inorganic N) were then combined at their respective optimum levels and tested for the symbiotic interaction between plants and VAM fungi. When the soil samples were amended with these nutrients, there was an increase in mycorrhizal activity and plant growth in the uneroded soil but not in the eroded soil. Inoculating the soil samples (amended with all the nutrients) with *G. aggregatum*, resulted in a significant increase in mycorrhizal activity and plant growth and the difference that existed between the unamended eroded and uneroded soils disappeared. Application of only basal nutrients did not influence mycorrhizal activity.

The results of these studies demonstrate the possibility of rehabilitating eroded soils by establishing effectively nodulated legumes through VAM inoculation and chemical amendments.

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INTRODUCTION

Erosion is defined as the wearing away of the land surface by running water, wind, ice or other geological agents, including such processes as gravitational creep. The impact of erosion on soil productivity is severe and has drawn the attention of many scientists. According to a recent report by the Worldwatch Institute, erosion of agricultural topsoil is a "quiet crisis". Almost half of the world's crop lands are losing topsoil at a rate that will lead to famines in some parts of the world unless the loss is curbed. The study estimates the world-wide loss of topsoil from crop lands at 25.4 billion tons a year. In the United States, 44 percent of the crop land is losing topsoil at excessive rates, with 1.7 billion tons permanently lost each year. The problem is becoming more alarming in certain parts of the world, especially in the tropics where there already exists a scaricity of both food and crop lands. In India alone, it is estimated that nearly 6 billion tons of soil is lost every year as a result of erosion.

Erosional soil losses are known to bring about unfavorable changes in soil structure, texture, bulk density and reduction in pH and in the contents of organic matter, nitrogen, available phosphorus, potassium and other nutrients (see Appendix A). Another detrimental influence of erosion is the lowering of biological activity, especially the loss in the activity of beneficial soil microorganisms such as vesicular-arbuscular mycorrhizal (VAM) fungi and rhizobia. All of these factors contribute to poor growth of plants in eroded soils. According to one study done in the tropics, every cm of topsoil removal is associated with a reduction of 76 Kg/ha in maize yield. It is thus important that we know more about eroded soils and try to rehabilitate them in order to improve their productivity and to reduce further erosion.

Vesicular arbuscular mycorrhizal fungi are important in improving plant growth through the increased uptake of nutrients, especially P. They are also believed to help plants establish on poor and eroded soils. Since eroded soils are low in nutrients and also in mycorrhizal fungi, the use of VAM fungi along with some chemical amendments would appear to be appropriate for rehabilitating such soils. Legumes are important source of dietary nitrogen in many parts of the world. Similarly, growing legumes in the eroded soil in the presence of VAM fungi and appropriate rhizobia is expected to improve nitrogen nutrition of plants through improved biological N_2-fixation.

The activity of VAM fungi is likely to be affected by soil properties. Changes in the chemical and nutritional properties of soil resulting from erosion, thus, may impose restrictions on the symbiotic activity of VAM fungi and rhizobia. Therefore, it is important to determine the levels of nutritional amendments that should be made for obtaining maximum benefits from VAM and rhizobial inoculation of eroded soils. The objectives of this investigation were:

1. Determine the influence of simulated erosion on the population and activity of vesicular-arbuscular mycorrhizal (VAM) fungi.
2. Evaluate the relative effectiveness of VAM species in improving the growth of cowpea and leucaena in an eroded soil.
3. Determine the optimum levels of phosphorus, lime, inorganic nitrogen, organic residue and molybdenum necessary for establishing effectively nodulated and mycorrhizal cowpea and leucaena in an eroded soil.
4. Determine the combined effects of nutrients identified above on the growth of mycorrhizal and effectively nodulated cowpea and leucaena in an eroded soil.

CHAPTER 1

REVIEW OF LITERATURE

Vesicular-arbuscular mycorrhizal fungi and their importance

The roots of most plants form symbiotic associations with a group of soil fungi known as vesicular-arbuscular mycorrhizal (VAM) fungi belonging to the family Endogonaceae. The symbiosis occurs under tropical, temperate and even arctic conditions (46). Growth responses of many plant species due to infection by VAM fungi have been shown to be largely due to better nutrient uptake, especially P (8,34,45,65,88). Moreover, VAM fungi are believed to be involved in the protection of plants against diseases (25,53), in the improvement of soil aggregation (91), enhancement of water uptake (68,80,81) and in the synthesis of hormone like substances (7).

Significance of VAM fungi to tropical legumes

Growth responses due to infection with VAM fungi is generally greater in legumes than in non-legumes. Asai (4), more than four decades ago, first demonstrated that legumes grew poorly and failed to nodulate in sterilized soil if they were not mycorrhizal. During the past few years, considerable attention has been given to the tripartite association among leguminous plants, rhizobia and VAM fungi. Vesicular-arbuscular mycorrhizal fungi play critical roles in this association because of the fact that they help the plants in the uptake of phosphorus that is essential and required in large amounts for growth as well as for satisfactory nodulation and N_2 fixation.

The dependence of plants on mycorrhizal infection is believed to be related to the extent of root hair development (10,11,89). Baylis (10,12) and St. John (89) hypothesized that plants with few or short root hairs may depend more on VAM fungi than plants with well-developed root hairs. There are, however, instances where plants with well-developed root hair systems could benefit from mycorrhizal infection (66). Plant physiological factors as well as root geometry of the particular plant species may be related to the degree of mycorrhizal dependency. The relatively high P requirement of N_2 fixation and their restricted root system make legumes a special case where they respond favorably to mycorrhizal infection (65). Crush (19) pointed out that tropical legumes were much more dependent on mycorrhiza for growth than temperate species and this difference seems to be related to the degree of root hair development. Increase in growth, nodulation and N_2-fixation of various leguminous crops due to infection with VAM fungi have been observed by many workers (5,6,9,16,37,47,76,94).

Erosion and its effect on soil properties

Erosion is defined as the "wearing away of the land surface by running water, wind, ice or other geological agents, including such processes as gravitational creep" (85, cited in 30). Erosion brings about significant changes in the physical, chemical and biological properties of soil which, in turn, affect soil productivity. With increase in topsoil removal, the bulk density of a soil increases and its content of organic matter, total N, available P and K decrease, apart from changes in soil structure, texture and pH (28,33,39,58,63,84). Habte (1985, Unpublished report) observed significant decrease in Ca, Mg and K contents of the Wahiawa soil at various levels of top soil removal ranging from 7.5-37.5 cm. The uptake of various macro and micro nutrients by Sesbania grandiflora (L.) Pers was also reduced significantly as a result of simulated erosion. The influence of top soil removal on the concentration of Mo (an impoprtant element for biological N_2-fixation) is not known and needs to be determined.

Biological properties of soil are also affected by erosion. Studies conducted by Habte (1985, Unpublished report) indicate that surface soil removal of > 15 cm resulted in a 10- and 100-fold decrease in the populations of cowpea and leucaena Rhizobium in the Wahiawa soil. The study further showed that the populations of soil fungi and protozoa were also reduced significantly by increasing levels of simulated erosion. There were, however, no significant changes in the populations of heterotrophic bacteria and actinomycetes. Eicker (29) also observed a decrease in fungal population with top soil removal.

VAM fungi in disturbed and eroded soils

Most of the work done regarding changes in the population of VAM fungi are in disturbed lands such as abandoned road beds, reclaimed mine spoils etc. (62,64,78). Reeves et al. (78) reported that more than 99% of the plant cover in the natural community was mycorrhizal, whereas less than 1% of the plant cover in the disturbed area (an abandoned road bed) was mycorrhizal. Moorman and Reeves (64) also reported that the percent infection in a disturbed abandoned road bed was 2% compared to 77% in an adjacent undisturbed soil, whereas Miller (62) noticed no VAM infection in plant species from a disturbed reclaimed spoil pile. These observations strongly indicate that the VAM population is significantly reduced as a result of land disturbances.

Habte (1985, Unpublished report) noted significant reduction in VAM infectivity of Wahiawa soil with increasing levels of simulated erosion. In another study, Redhead (77) observed a decrease in the number of mycorrhizal spores with top soil removal. Schwab and Reeves (84) measured the changes in VAM inoculum potential in a soil profile using a bioassay technique. The inoculum potential was significantly reduced below 30 cm depth and approached zero at less than 1 m depth. Their explanation for decrease in mycorrhizal inoculum potential with depth was the reduction in numbers of fungal propagules although the cause of inhibition due to changes in soil chemical factors was not ruled out. Powell (73) also used a bioassay technique to assess the effect of soil erosion on mycorrhizal propagule numbers in soils that were collected from different eroded sites in New Zealand. He found very few propagules in those soils although nothing was known about the nature and extent of erosion. So, the information available on VAM-erosion interaction is still very rudimentary.

Response of plant to mycorrhizal inoculation in eroded soils

Although in some instances mycorrhizal fungi have been shown to improve revegetation in disturbed sites such as coal mine spoils, strip mines etc. (20,21,22), very little is known about the role of VAM fungi on the growth of plants in eroded soils. In a pot experiment, Hall et al. (43) observed that reintroduction of VAM fungi in eroded soil increased the growth of lotus and white clover. In another experiment conducted in the field, Hall (42) observed marked growth increase of lotus in eroded soil inoculated with VAM fungi using infested soil pellets. Increase in plant growth was attributed to the introduction of VAM fungi and also pellets to soil. Similar increase in growth in an eroded soil has also been noted by Powell (73). In a pot experiment he observed a 1- to 12-fold increase in the growth of clover due to inoculation of eroded soils that varied in P content as well as in mycorrhizal infectivity. Results of this study emphasize the importance of inoculating eroded soils with efficient strains of VAM fungi.

The works mentioned above were, however, done on temperate soils, so the results obtained can not be directly related to tropical soils that have different properties and characteristics. More work is, thus, needed in this area. Furthermore, the response of only few plant species to VAM inoculation in eroded soils is known so far. It is also not known precisely what factors in eroded soil are detrimental to VAM fungi or how VAM fungi can help plants establish in eroded soils.

Relative performance of VAM species

VAM fungi differ in their abilities to stimulate P uptake and plant growth (1) and some soils are populated only by relatively inefficient strains (75). Consequently,there is interest in the possibility that plant growth might be stimulated by inoculating soils with efficient strains of VAM fungi. Various studies have shown that the performance of VAM fungi with regard to root colonization, development and spread of infection and relative effectiveness differ from one another in different environments and host plants (3,15,26,48,72,83,92,93,96,97). None of these studies was done on eroded soils. Powell (73), however, reported that G. tenuis was more efficient than E3 in increasing the growth of clover in the eroded soil although, nothing was defined about the degree of erosion and changes in soil properties. So it is important to determine the relative performance of VAM species in soils subjected to simulated erosion.

Effect of phosphorus on VAM activity

Increasing the P supply frequently decreases the percent of root length infected by VAM fungi and their functions (1,2,49,55,59,61,71,79,83,88,92). Davis et al. (24) has, however, reported the detection of high P tolerant VAM fungi. This could be of great importance in increasing the efficiency of VAM fungi in high P soils. The effect of P supply in decreasing the proportion of roots that are mycorrhizal is believed to arise from the effects of P supply in stimulating root growth more than the ability of the fungus to infect (1,14). On the other hand, Bolan et al. (13) observed that increasing P supply can increase the infection of plant roots by VAM fungi. The levels of P employed were, however, not well defined and so can not be compared with the findings of other investigators (1,14). Bolan et al. (13) have suggested that the increase in percent root length infected with increasing P supply is not a direct effect of P supply on root growth but rather a direct effect of P supply on the growth of the fungus itself or an indirect effect on the fungus mediated by altered plant metabolism as a result of P supply. Same et al. (82) also noticed increase in percent root length infected due to small addition of P to severely deficient plants.

Powell (74) has pointed out that the effect of VAM fungi should be tested at a series of phosphate levels in order to select the P doses optimum for the mycorrhizal symbiosis. Yost (98) tested the effect of 10 P concentrations on leucaena grown in non-fumigated Wahiawa soil and concluded that the concentration of P for best growth was 0.05 mg/l soil solution. To derive maximum benefits from dual inoculation with Rhizobium and VAM fungi, addition of a small amount of P has been shown to be important for cowpea and pigeon pea (60). Most of these works were based on the amount of P applied to the soil rather than on the amount of available P in the soil solution. Since soils differ in P sorption capacities (32,56), they would contain different amounts of available P in soil solution for the same amount of applied P. Hence the results can not be compared for different soils. It is, thus, important that P should be applied on the basis of P sorption curve. Habte and Manjunath (40), however, applied P to soil based on the P-sorption characteristic to obtain a range of available P in the soil solution. Results of their work indicate the presence of an optimum level of soil solution P for mycorrhizal activity.

Very little information is available on the influence of P on mycorrhizal symbiosis in eroded soils. Since eroded soils are deficient in P and also have a low VAM population, it is likely that these soils could easily be rehabilitated by growing legumes through the use of VAM fungi and P application. It is, therefore, important to determine the optimum level of P in eroded soils for obtaining maximum mycorrhizal benefits.

Effect of pH on VAM activity

VAM fungi often show an adaptation to soil pH. Both spore germination and mycorrhizal development by different fungal species can be significantly affected by variation in soil pH (23,38,41,48). Skipper and Smith (87) observed that when soybean cultivars were inoculated with *Gigaspora gigantea* and *Glomus mosseae*, the response of the specific cultivar-fungal association was dependent on soil pH. Huang et al. (52) studied the effect of 3 mycorrhizal isolates on the growth of *Leucaena leucocephala* at 3 pH levels and observed that the best response to inoculation was obtained when *G. fasciculatum* was used as inoculum at a soil pH of 5.7. Their results indicate some degree of soil pH-endophyte specificity.

Since pH tends to decrease with topsoil removal (63), its influence on the VAM symbiosis should be determined and if adverse, the situation be rectified either by liming or through the selection of VAM endophytes adapted to low pH.

Effect of inorganic nitrogen on nodulation and VAM activity

Legumes usually do not need N fertilizers when they are adequately nodulated, but when present in excess amounts, these compounds are deleterious to nodulation and N_2-fixation (35,36 cited in 8). The requirement of a small amount of nitrogen as a starter N is important in legumes under the conditions of nitrogen deficiency in soil. According to Kanehiro et al. (57) small amount of available N in soil at the right time could stimulate N_2-fixation, and large amounts are harmful for biological N_2-fixation. They suggested that the addition of starter N is beneficial in highly weathered, low organic matter, acid soils. Munns (67) showed that 0.02 to 0.05 mM N concentration in solution inhibits nodulation at the begining but not in the later stages of plant growth. Ezedinma (31) working with topsoil from four locations in Nigeria observed that available N upto 100 ppm applied as KNO_3 increased the number and weight of nodules of cowpea and the amount of N fixed. Higher levels tended to give lower results. He suggested that small doses of fertilizer N should be applied to cowpea at planting. Summerfield et al. (90) grew cowpea in medium containing peat, sand and crushed grit in the ratio of 5:1:1 and observed better growth and nodulation when N was applied at 30 or 60 ppm rate. More recently, Eaglesham et al. (27) grew cowpea and soybean in polystyrene pots filled with silica sand with charcoal chips at the bottom and concluded that applied N in the range of 30 to

180 mg N/plant may have synergistic effects on N_2-fixation by vigorously growing cowpeas and soybeans.

In contrast to the works mentioned above, Huxley (54) reported that the benefits obtained from starter N is not as much as has been documented. The results of his experiment using radioactive N show that only about 1/5th or less of the starter N applied was taken up by the plant. The auther points out that with such small amounts of N utilized, it is not surprising that effects on growth and yield were insignificant. The auther further suggested that a single starter dose, even at the low levels used, is probably not as helpful to nodule development and activity as that of a continously administered low supply. Most of the work on starter N has been done with culture solutions where the initial N levels were zero or negligible. In soils, where there already exists some inorganic N, it is difficult to define starter N levels unless the inorganic N content of soils is known and taken into consideration. The role of starter N may be more important in eroded soils because of their low N content.

There is scarcity of information on the interaction between inorganic N and the tripartite association between legumes, rhizobia and VAM fungi. Hayman (44) showed that N fertilizers had a large negative effect on mycorrhizal populations and plots not given N contained 2 to 7 times more endogonaceous spores and 2 to 4 times more VAM infection than plots given N. Similar suppression of VAM infection (in addition to nodulation) by fertilizers has also been observed in clover (17,18). Addition of NH_4 was more deleterious than that of NO_3 (17). On the other hand, Hepper (50) observed that application of increasing amounts of NO_3 resulted in higher levels of VAM infection in the roots of lettuce inoculated with G. mosseae. She stressed that the ratio of applied N to P is important in determining mycorrhizal infection. Based on her results, she predicted that an applied N/P ratio in excess of 15 would be required to obtain reasonable levels of infection in lettuce under the circumstances under which the experiment was carried out. It is possible to maintain such ratios in artificial media but not in soils where so many factors are involved in determining the availability of applied nutrients. Eroded soils being low in N may impose some constrains on the development of infection and legume establishment. The right amount of inorganic N application for maximum mycorrhizal symbiosis and nodulation in the eroded soil, therefore, needs to be determined.

Influence of organic matter on VAM activity

Organic matter influences soil structure, pH, nutrient and water-holding capacity, all of which directly or indirectly may influence VAM infection and its effectiveness (34). According to Sheikh et al. (86, cited in 34), endogoneous spore population seems to be closely coorelated with the level of organic matter content of soils. Maximum spore numbers were recovered from soils containing 1 to 2% organic matter, and spores were sparse in soils having less than 0.5% organic matter.

It has been suggested that VAM fungi might be able to exist saprophytically on decaying root fragments and other organic matter. This suggestion came from the observations that the mycelium associated with roots infected with VAM fungi often grow around organic materials (69). Further evidence for saprophytic growth of VAM fungi has been given by Warner and Mosse (95) who showed that hyphae could grow through soil and establish a base from which they could independently infect a host plant. Ocampo and Hayman (70) observed that inoculum stored at ambient temperature has a higher inoculum potential than that stored at 2 C which has been interpreted as suggesting the possibility of saprophytic growth. Hepper and Warner (51) observed increase in growth and root activity of clover due to inoculation with G. mosseae when the soil was amended with organic material. Based on their results the authors concluded that VAM fungi could grow saprophytically in soil but did not give a possible mechanism that might be involved.

Thus, from these studies it appears that organic matter in soil may play a role in the infection process. No information is, however, available on the role of organic matter in establishing mycorrhizal association in eroded soils.

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CHAPTER 2

GENERAL METHODS AND MATERIALS

Soil used

The soil used in this study (Wahiawa soil series) is classified as a clayey, kaolinitic, isohyperthermic family of Tropeptic Eutrustox which was collected from Poamoho Experiment Station of the Hawaii Institute of Tropical Agriculture and Human Resources (HITAHR), Island of Oahu. This soil is developed in residuum and old alluvium derived from basic igneous rock. It occurs in nearly level to moderately steep landscape at an elevation range of 150 to 365 m. Rainfall amounts to 1000 to 1500 mm annually, and the mean annual soil temperature is 22 C (1). The clay fraction of the soil contains kaolinite, maghemite, gibbsite, halloysite, geothite, haematite and anatase (Dr. R.C. Jones, University of Hawaii, Personal Communication).

Simulation of erosion

Erosion was simulated by removing the top 30 cm of soil. The remaining soil was considered eroded soil. Sampling was done to an additional 15 cm of depth. Uneroded soil was obtained from an adjacent undisturbed site which was also sampled to a depth of 15 cm.

Soil preparation

The soil samples were air-dried under shade for one week after which time they were crushed to pass a sieve with 4 mm openings. They were then mixed thoroughly and stored in tightly covered plastic trash containers until used. Two kg portions of soil (oven-dry basis) were transfered to 15 cm diameter by 15 cm deep plastic pots.

Plant species and seed treatments used

The plant species used in this study were: Leucaena leucocephala (Lam.) de Wit, var. K-8 (leucaena) and Vigna unguiculata (L.) Walp, var. California Black Eye (cowpea). Seeds of leucaena were obtained from the NifTAL project, Maui and that of cowpea were purchased from Down-to-Earth Natural Foods Inc. (2525 South King Street, Honolulu, Hawaii). In the text, figures and tables to follw, only the common names of these leguminous plants will be used instead of their scientific names.

Healthy seeds of uniform size were selected for conducting experiments. Seeds of leucaena were scarified with concentrated sulfuric acid for 20 minutes and rinsed 6 times with sterile water. Cowpea seeds were surface sterilized with 3% hydrogen peroxide solution for 3 minutes and rinsed 6 times with sterile water.

Inoculation of seeds with Rhizobium

Bradyrhizobium sp. strain TAL 209SR and Rhizobium sp. strain 1145SR were used to inoculate the cowpea and leucaena seeds, respectively. Both cultures were obtained from Dr. M. Habte, Department of Agronomy and Soil Science, University of Hawaii. Stock cultures of the bacteria were maintained on yeast mannitol agar (YMA) slants at 4 C. Four-day and 6-day old cultures of Rhizobium sp. (strain 1145 SR) and Bradyrhizobium sp. (strain TAL 209 SR), respectively, grown on YMA slants at 30 C were used for inoculating seeds. For the purpose of inoculation, the cultures were suspended in sterile saline and applied to scarified seeds. To give protection to rhizobial cultures, the inoculated seeds were coated with a finely-ground sterile peat. The seeds were pre-germinated in water agar (0.9%) at 30 C for 2 days before planting.

Vesicular arbuscular mycorrhizal fungi used

The vesicular arbuscular mycorrhizal (VAM) fungi used in this study were Glomus aggregatum Schenck & Smith emend. Koske, G. mosseae (Nicol. & Gerd.) Gerdemann & Trappe, and G. etunicatum Becker & Gerd. Glomus aggregatum was obtained from Dr. M. Habte, Department of Agronomy and Soil Science, University of Hawaii; G. mosseae and G. etunicatum were obtained from Dr. N.C. Schenck, Department of Plant Pathology, University of Florida. For increasing the cultures, about 20 Kg of sand (manufactured by crushing basaltic rock) were placed in 71 X 42 cm plastic bags and fumigated in a gas-tight chamber twice at an interval of 10 days by exposing them to 48 g of methyl bromide and 1.0 g of chloropicrin per chamber (volume = 0.712 m^3) for 5 days. After fumigation, the bags were removed from the chamber and allowed to stand for two weeks to dissipate the fumigants from the soil. Six to eight surface sterilized corn (Zea mays L.) seeds were planted in each bag and the plants were grown in the greenhouse under natural light. During the period of plant growth, each bag was amended with 200 ml portions of a N- and P-free nutrient solution (4) three times a week for two months. Watering was stopped after about three months and the plants were allowed to dry. After harvesting the corn, the shoots and large roots were removed and the remaining sand containing bits of hyphae, pieces of infected roots and spores was stored in the air-dried state to serve as experimental crude inoculum. VAM inoculum was mixed thoroughly with the soil contained in each pot designated for VAM inoculation. The quantities of inoculum used were 50, 71, and 96 g for G. aggregatum, G. mosseae and G. etunicatum, respectively, per pot. The quantities of inoculum applied for different species of VAM fungi were obtained from a preliminary experiment in order to get comparable levels of effective propagules. The uninoculated controls received washings of the crude inoculum (after passing it through Whatman No. 1 filter paper) and sterile sand instead of inoculum.

Basal nutrient application

A basal nutrient treatment was applied to all soils as follows: The phosphorus sorption isotherm (2) was used to establish a P level of 0.026 mg/l in the soil solution (except for the experiment in which phosphorus was a variable). Lime was added in the form of $Ca(OH)_2$ to raise the pH of soil to 6.0 for cowpea and 6.5 for leucaena (except for the experiment in which lime was a variable). pH values of 6.0 and 6.5 gave final Ca concentrations of 1339 and 1898 ppm, respectively, in the uneroded and 1476 and 1955 ppm, respectively, in the eroded soil. Nitrogen was added at the rate of 150 ppm in the form of NH_4NO_3 (except for the experiment in which nitrogen was a variable). Potassium and magnesium were added to the soil samples in the form of K_2SO_4 and $MgSO_4 \cdot 7H_2O$, respectively, to obtain concentrations of 250 and 212 mg/Kg soil, respectively. A solution containing $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $Na_2B_4O_7 \cdot 10H_2O$ and $NaMoO_4 \cdot 2H_2O$ was also added to the soil at the rate of 10, 5, 0.1, and 0.5 mg, respectively, of Zn, Cu, B and Mo per Kg of soil.

The soil was limed first and allowed to equilibrate at 60% of water holding capacity for 2 weeks. Phosphorus was then added and mixed well with the soil in each pot and allowed to equilibrate for a further period of 6 days at about 60% water holding capacity. Finally, all the remaining nutrients were added in the form of solution just before planting.

Planting seeds and other procedures

Three pregerminated seeds were planted in each pot. After emergence, the seedlings were thinned to two plants per pot. Treatments were arranged on glasshouse benches in a randomized complete block design (RCBD) with three replicates per treatment (except the experiment in chapter 4 which had four replicates per treatment). The plants were grown under natural light (21 51'N and 156 22' W) in the University of Hawaii Agronomy and Soil Science glass house. Pots were watered with deionized or distilled water as needed to maintain the soils at about 60% of water holding capacity. Leucaena plants were sprayed with Cygon [active ingredient:

Dimethoate-(0,0-dimethyl S-(N-methyl-carbamoyl-methyl) phosphorodithionate, 23.4%] as needed to control the "leucaena psyllid" (*Heteropsylla cubana* Crawford). Cowpea plants were sprayed with powdered sulfur for the control of powdery mildew (caused by *Oidium* sp.).

Sampling of subleaflets or leaf discs for phosphorus determination

Every 5 days starting from 12 days after planting (DAP) until harvest, subleaflet or leaf disc samples were taken from leucaena and cowpea plants, respectively, for P determination. The third subleaflet from the base of the youngest fully expanded leucaena leaf was collected while leaf discs were removed from the youngest fully opened cowpea leaves using a cork borer of 0.8 cm diameter.

Plant growth and nodulation measurements

After harvest, roots were washed carefully with water to remove all the soil particles with minimal loss of roots and nodules. Nodules were then separated from roots. Shoot, root and nodule dry weights were recorded after oven-drying at 70 C for 48 hours.

Root colonization by VAM fungi

To determine the extent of root length colonized by VAM fungi, 0.3 g portions of fresh roots were sampled randomly from each pot. The roots were cleared and stained according to a slight modification of the method described by Phillips and Hayman (6). The modification was that I used 0.15% acid fuchsin in lactic acid instead of 0.05%

trypan blue in lactophenol. Percent root colonization was determined by the grid line intersection method (3).

Plant tissue analyses for phosphorus

Dried shoot and root samples were finely ground (<1 mm) and stored in plastic containers for nutrient analyses. The molybdate blue method (5) was used to determine P content of all tissues after dry ashing at 500 C for three hours.

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CHAPTER 3

EFFECT OF SIMULATED EROSION ON THE POPULATION AND ACTIVITY OF VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI

INTRODUCTION

Erosion brings about significant changes in the physical, chemical and biological properties of soil as the topmost surface layers are lost during the process. These changes include the loss of many essential nutrients and soil microorganisms that are important to plant growth. The consequence is a reduction in soil productivity.

In attempts to improve the productivity of eroded soils, various investigators have studied in great detail the changes in the chemical and physical properties of soil brought about by erosion. In contrast, little is known about the effect of erosion on soil biological properties. In order to rehabilitate eroded soils it is equally important to consider the detrimental efffects of erosion on both the nutrient content and biological properties of soil, especially the effects on the activity of vesicular-arbuscular mycorrhizal (VAM) fungi and their symbiotic association with plants.

The objective of this study was to determine the influence of simulated erosion on the population and activity of VAM fungi.

MATERIALS AND METHODS

For the enumeration of VAM spores, the soil pH was adjusted to 7.0, moistened to about field capacity and allowed to equilibrate for 1 day. Spores were separated into different size groups by the wet seiving and decanting technique of Gerdemann and Nicolson (1) using sieves of 0.0594, 0.0297, 0.0150, 0.0104 and 0.0053 cm aperture size. Total spore numbers were determined by transfering all the spores from each sieve into a fine nylon mesh which was then placed in a petri dish marked with horizontal lines 1 cm apart. Spores were then counted under a stereo microscope at 40X magnification.

Enumeration of infective propagules of VAM fungi was determined by the "Most Probable Number" method described by Porter (5). For making soil dilutions, 200 g portions of a fumigated 1:2 (by weight) sand:soil mixture (diluent) were placed in plastic bags (size = 25.5 X 12.5 cm). Fifty g of the soil sample (which is to be tested) was added and mixed with the sand-soil mixture contained in plastic bags. This gave a dilution of 5^{-1}. Soil dilutions used for determining the MPN were 5^{-2}, 5^{-3}, 5^{-4} and 5^{-5} with 5 replications per dilution. The supporting medium used was a fumigated sand:soil mixture (1:2). Four hundred g of this medium was placed in each dibble tube (size = 25 cm height, 6.2 cm internal diameter at top) and mixed well with 30 g of the diluted soil. Cowpea was grown in the dibble tubes in the greenhouse for 5 weeks. Roots were then examined for mycorrhizal colonization. The extent of VAM colonization of roots of native vegetation was determined by the grid line intersection method (2) after clearing and staining roots according to a slight modification of the method described by Phillips and Hayman (4). The modification was that I used 0.15% acid fuchsin in lactic acid instead of 0.05% trypan blue in lactophenol.

RESULTS AND DISCUSSION

Simulated erosion resulted in a significant decrease in VAM infectivity of soil (Table 3.1). Since VAM fungi form a symbiotic association with plant roots, their population and activity are likely to be confined to that region of soil where plant roots are concentrated. VAM spore numbers were reduced by 78% due to topsoil removal which was also associated with a reduction in plant roots (see Appendix A). The total number of mycorrhizal spores does not give a true picture of mycorrhizal activity since all the spores may not be viable or effective. For this reason a count of infective propagules of VAM fungi was made.

The number of infective propagules in soil was reduced by 7 folds by removal of topsoil. The number of infective propagules counted was 3 to 4 times lower than the number of total spores in both soil samples, indicating that a substantial portion of the spores was not viable. Apart from reduced plant root density, reduction of spore numbers with soil depth could also be related to the changes in soil properties associated with topsoil removal. Germination of spores and root colonization by VAM fungi have been reported to be influenced by

TABLE 3.1. Influence of simulated erosion on mycorrhizal infectivity of soil[a]

Soil	Spore number per g soil	Infective Propagule Number per g soil	Colonization of roots of native vegetation (%)	Length of colonized roots per g soil
Uneroded	407a	140a	63.9a	12.96a (cm)
Eroded	88b	20b	27.5b	0.54b (cm)

[a]Means followed by the same letter within a column are not significantly different at the 5% level.

soil nutrient contents and pH (3). Similar results were obtained by Redhead (6) and Schwab and Reeves (7). The extent of VAM colonization of roots of native vegetation in the eroded soil was less than half compared to that in the uneroded soil. When the colonization of roots was expressed as the length of roots colonized per g of soil, the value obtained was 96% lower in the eroded than in the uneroded soil. The excessive reduction in root length colonized by VAM fungi was a result of the reduction in native roots as a result of simulated erosion.

Hence, simulated erosion caused a significant reduction in the population and activity of VAM fungi in soil which must be restored by inoculating the soil with VAM fungi in order to increase its productivity.

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CHAPTER 4

RELATIVE EFFECTIVENESS OF THREE VAM SPECIES ON THE GROWTH OF NODULATED LEGUMES IN AN ERODED SOIL

INTRODUCTION

Vesicular-arbuscular mycorrhizal (VAM) fungi are known to improve plant growth through increased uptake of nutrients (5,8) but they differ in their abilities to do so (1,4,14). Different species of VAM fungi have been observed to perform differently in different soils (3,7) and in association with different host plants (11,12). Since some soils are populated only by relatively inefficient strains of VAM fungi (13), there is interest in the possibility of stimulating plant growth by inoculating these soils with efficient VAM strains. Plants grown on eroded soils could similarly benefit from such an inoculation because eroded soils are deficient in many nutrients, especially P, and VAM fungi might improve the P nutrition in these soils (10). However, there is very little experimental evidence to support the hypothesis that inoculating eroded soils with VAM fungi will improve plant growth. The objective of this study was to evaluate the relative effectiveness of three VAM species in improving the growth of cowpea and leucaena in an eroded soil.

MATERIALS AND METHODS

The mycorrhizal species used in this experiment were Glomus aggregatum, G. mosseae and G. etunicatum. Enumeration of infective propagules of the inocula was done as described in Chapter 3. The inocula for different species of VAM fungi was applied in such a way so as to get comparable levels of effective propagules.

Treatments consisted of eroded or uneroded soil, uninoculated or inoculated with G. aggregatum, G. mosseae, or G. etunicatum. The soils were not amended with fertilizers. The host plants used were cowpea and leucaena. Leaf disc or subleaflet samples were taken for P determination every 5 days beginning at 12 days after planting (DAP) until harvest. Cowpea and leucaena were grown for 52 and 57 days, respectively. At harvest, measurements of colonization of roots by VAM fungi, shoot and root dry weight, nodulation, and P content of shoots were determined.

RESULTS

Cowpea. The extent of colonization of cowpea roots by different VAM species is shown in Fig. 4.1. Inoculation significantly improved colonization of cowpea roots by VAM fungi in both soils. The extent of colonization was, however, greater when cowpea was grown in the uneroded soil rather than in the eroded soil. The VAM species did not differ significantly from each other in their ability to colonize

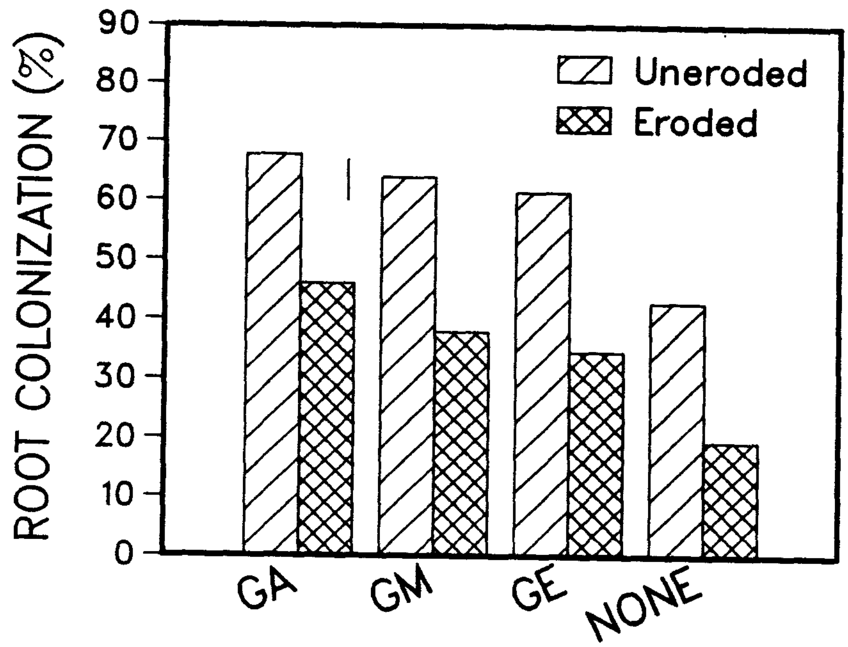

FIG. 4.1. The influence of VAM inoculation on the extent of colonization of roots of cowpea grown in uneroded or eroded soil. Vertical bar represents LSD at the 5% level. GA = G. aggregatum, GM = G. mosseae, GE = G. etunicatum.

roots of cowpea grown in the uneroded soil. In the eroded soil, cowpea grown in association with *G. aggregatum* had a significantly higher level of root colonization than cowpea grown in association with the other two VAM species.

Phosphorus content of cowpea leaf discs, in general, decreased initially in both the eroded and uneroded soil from about 4 g to 3 g/leaf disc and then increased to about the original level at 32-37 DAP (Fig. 4.2). Inoculation of the uneroded soil with *G. aggregatum* significantly increased mycorrhizal activity measured in terms of P content of leaf discs from 37-42 DAP. Similar results were obtained when mycorrhizal activity was monitored in terms of the P concentration of leaf discs [Fig. B.1 (Appendix B)].

Shoot P content of cowpea was significantly increased due to inoculation of the uneroded soil with VAM fungi (Fig. 4.3). The VAM fungi tested did not differ from each other in their abilty to increase shoot P content. On the other hand, there was no effect of inoculation in the eroded soil. Shoot P content of cowpea grown in the uneroded soil was significantly higher than those grown in the eroded soil.

Inoculation of the uneroded soil with VAM fungi increased nodule dry matter yield of cowpea (Fig. 4.4). The increase observed in this soil was in the order of: uninoculated < *G. etunicatum* < *G. mosseae* < *G. aggregatum*. Inoculation of the eroded soil did not result in an increase in nodule dry matter production.

There was a significant increase in shoot dry matter production of cowpea due to VAM inoculation in the uneroded soil but not in the

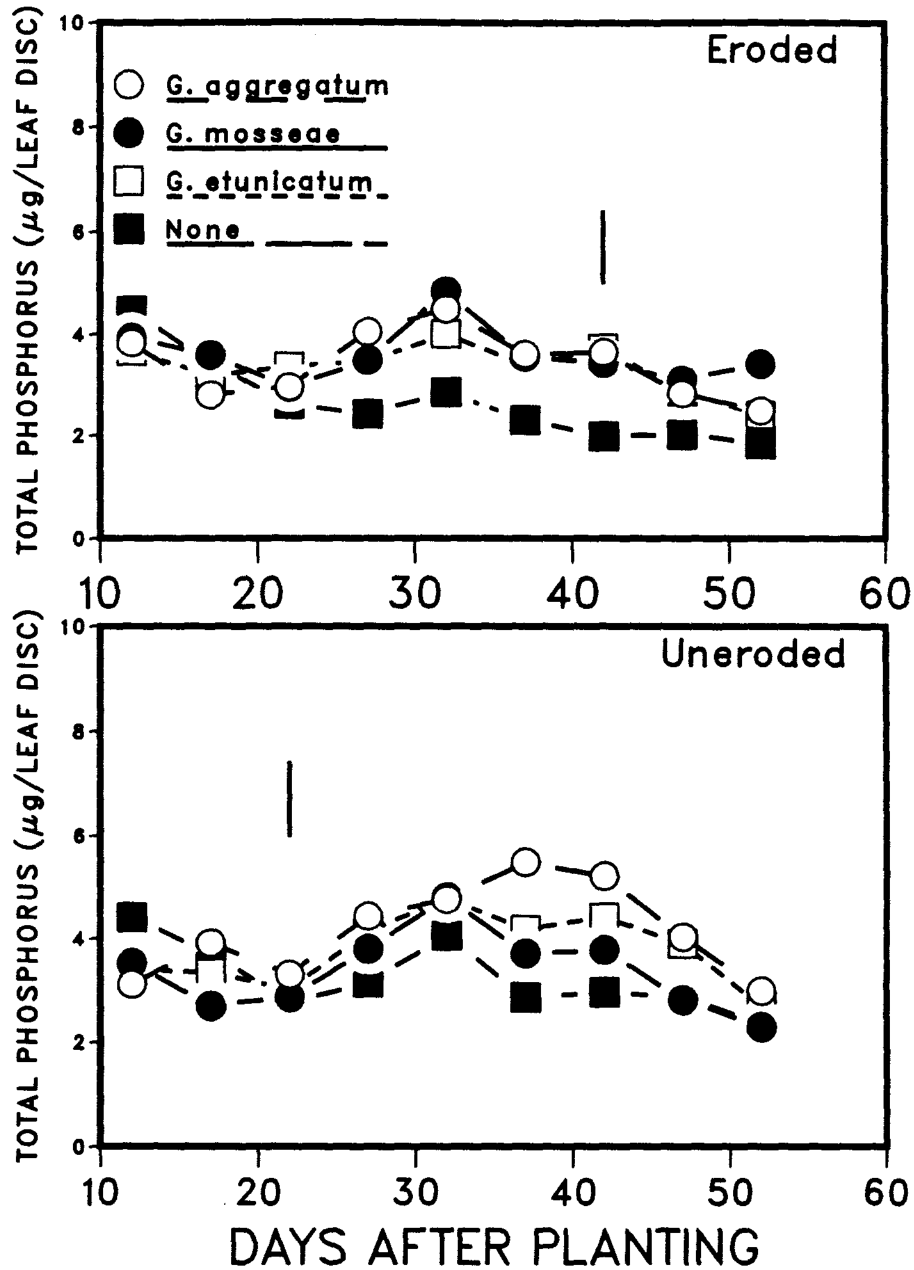

FIG. 4.2. The influence of VAM inoculation on the development of mycorrhizal effectiveness in cowpea grown in eroded or uneroded soil. Vertical bars represent LSD at the 5% level.

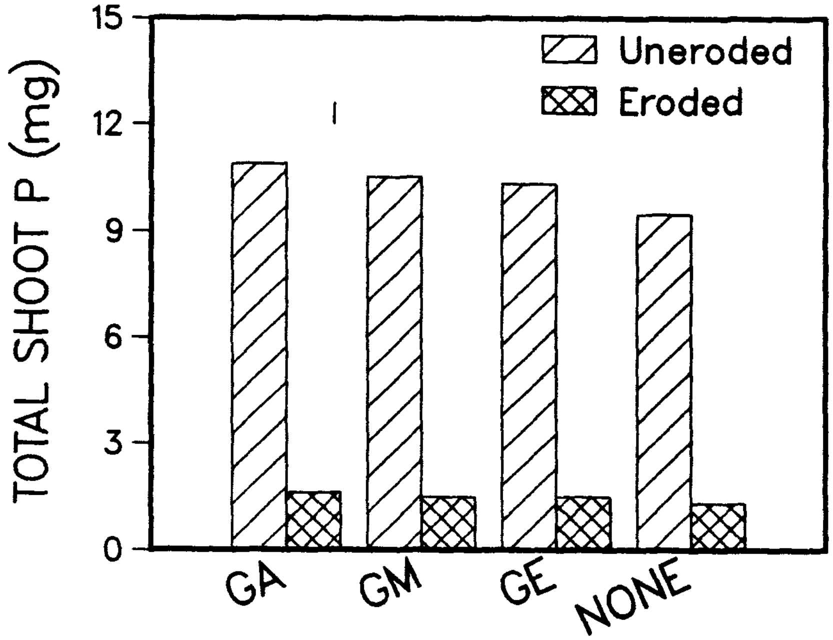

FIG. 4.3. The influence of VAM inoculation on shoot P content of cowpea grown in uneroded or eroded soil. Vertical bar represents LSD at the 5% level. GA = G. aggregatum, GM = G. mosseae, GE = G. etunicatum.

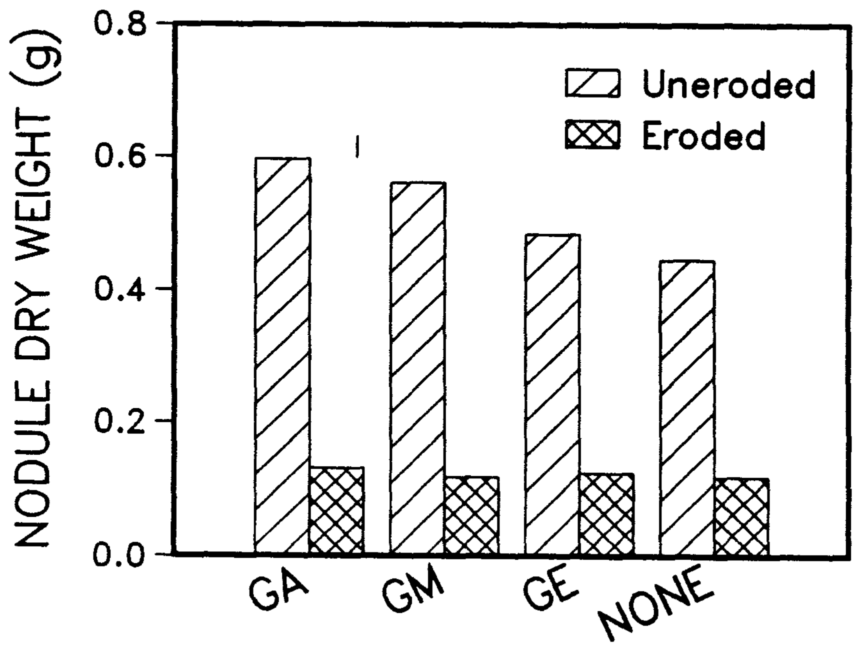

FIG. 4.4. The influence of VAM inoculation on nodule dry matter production of cowpea grown in uneroded or eroded soil. Vertical bar represents LSD at the 5% level. GA = G. aggregatum, GM = G. mosseae, GE = G. etunicatum.

eroded soil (Fig. 4.5). The shoot dry matter production of cowpea grown in the uneroded soil inoculated with G. aggregatum was similar to that inoculated with G. mosseae but higher than the one inoculated with G. etunicatum. Cowpea grown in the uneroded soil had significantly higher shoot dry weight than when grown in the eroded soil. Root dry matter production of cowpea grown in the uneroded soil increased only when inoculated with G. aggregatum while there was no increase due to inoculation in the eroded soil (Fig. 4.5).

Leucaena. Inoculation of both eroded and uneroded soils with different species of VAM fungi significantly increased the extent of colonization of leucaena roots (Fig. 4.6). The infectivity of the VAM species tested decreased in the order of G. aggregatum > G. mosseae > G. etunicatum in the uneroded soil and G. aggregatum = G. mosseae > G. etunicatum in the eroded soil. The extent of colonization of roots was significantly lower in the eroded soil than in the uneroded soil.

Figure 4.7 shows the changes observed when mycorrhizal activity was measured in terms of P content of leucaena subleaflets as a function of time. Mycorrhizal activity decreased initially and then stabilized at a level that was lower than the initial one. There was no significant increase in mycorrhizal activty in leucaena in either soil as a result of VAM inoculation. However, mycorrhizal activity observed in the uneroded, uninoculated soil was consistently low compared to that in the inoculated soils. The trends were similar when mycorrhizal activity was monitored in terms of the P concentration of subleaflets [Fig. C.1 (Appendix C)].

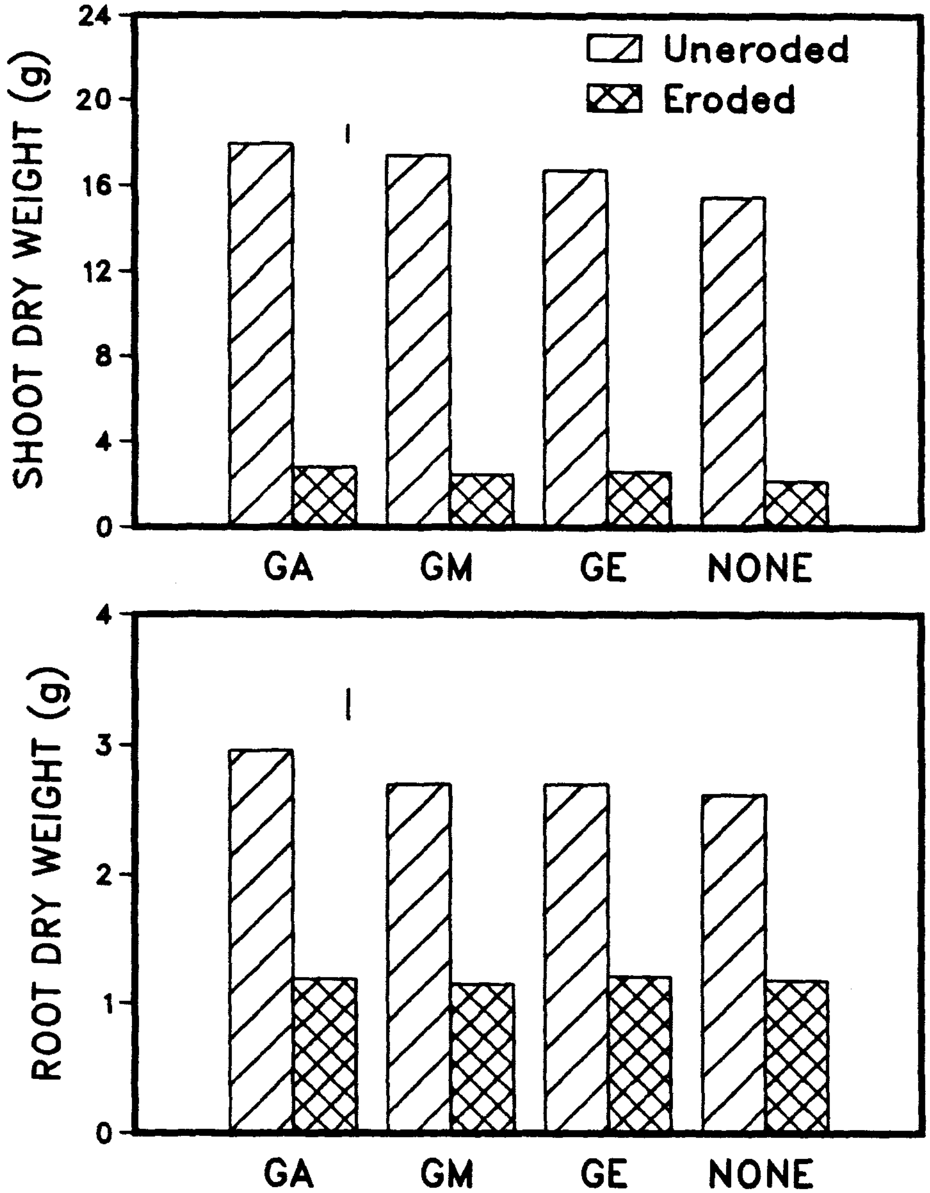

FIG. 4.5. The influence of VAM inoculation on dry matter production of cowpea grown in uneroded or eroded soil. Vertical bars represent LSD at the 5% level. GA = *G. aggregatum*, GM = *G. mosseae*, GE = *G. etunicatum*.

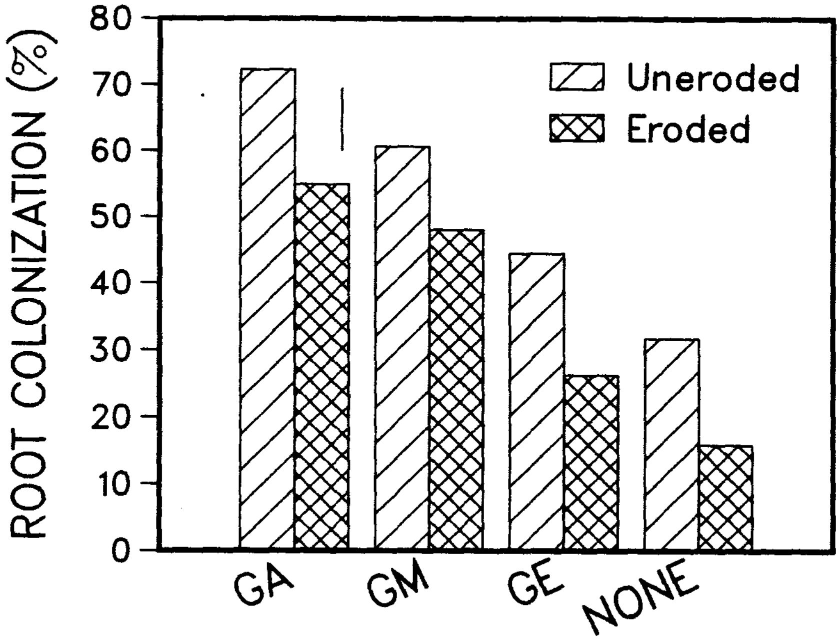

FIG. 4.6. The influence of VAM inoculation on the extent of colonization of roots of leucaena grown in uneroded or eroded soil. Vertical bar represents LSD at the 5% level. GA = *G. aggregatum*, GM = *G. mosseae*, GE = *G. etunicatum*.

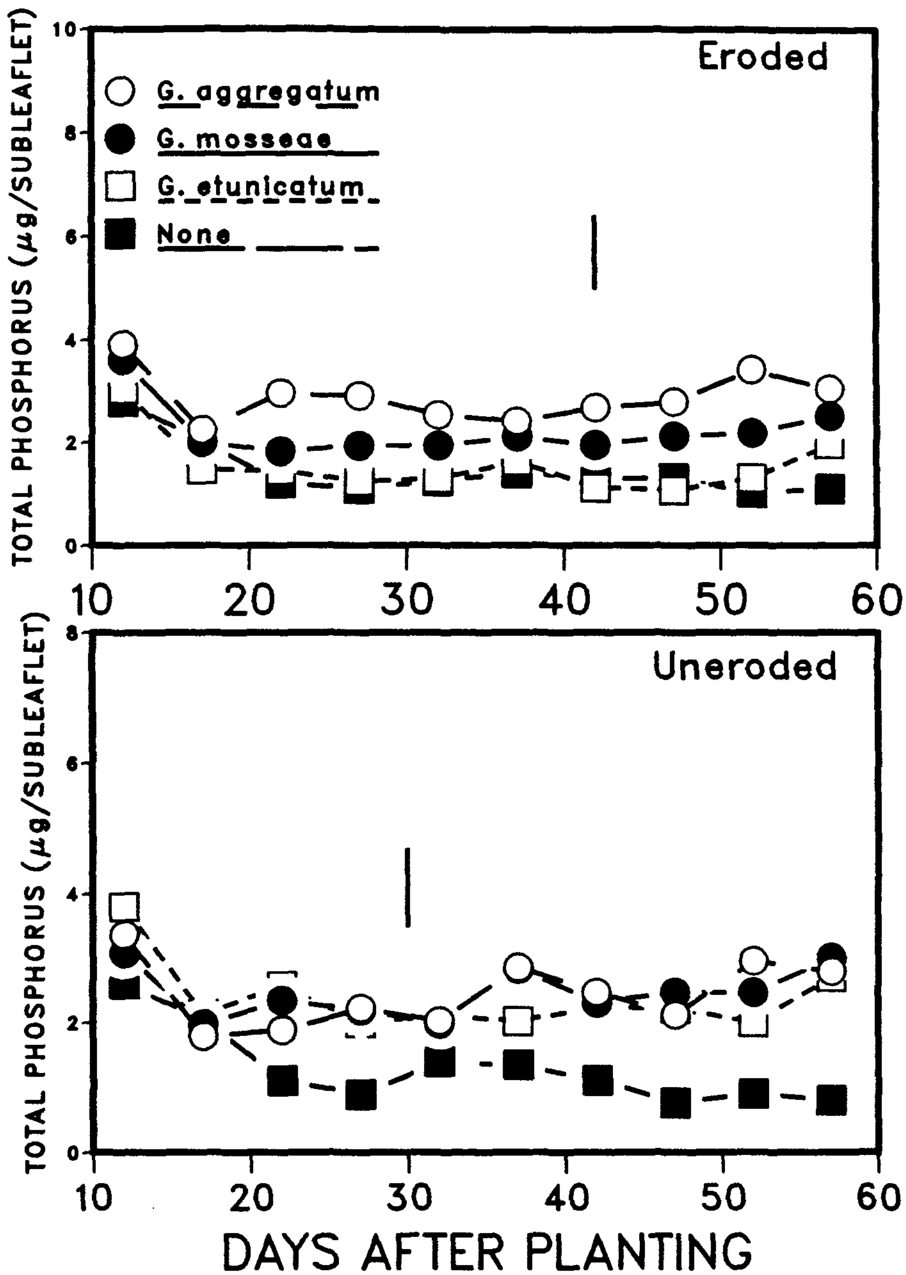

FIG. 4.7. The influence of VAM inoculation on the development of mycorrhizal effectiveness in leucaena grown in eroded or uneroded soil. Vertical bars represent LSD at the 5% level.

Inoculation of the uneroded soil with VAM fungi increased the Shoot P content of leucaena significantly (Fig. 4.8). The trend of shoot P content when leucaena was grown in the uneroded soil inoculated with different VAM species decreased in the order of _G. aggregatum_ > _G. mosseae_ > _G. etunicatum_ > uninoculated. No change in shoot p content was observed in the eroded soil due to VAM inoculation.

The changes in nodule dry weight values of leucaena associated with mycorrhizal inoculation are depicted in Fig. 4.9. Inoculation of the uneroded soil with _G. aggregatum_ or _G. mosseae_ resulted in a significant increase in nodule dry matter production compared to that of the uninoculated control, whereas inoculation with _G. etunicatum_ did not increase nodule dry matter yield. Of the three VAM species tested, _G. aggregatum_ was associated with the highest nodule dry matter yield in the uneroded soil. Vesicular-arbuscular mycorrhizal inoculation did not influence nodule dry matter production in the eroded soil.

The effect of VAM inoculation on dry matter production of leucaena is summarized in Fig. 4.10. Vesicular-arbuscular mycorrhizal inoculation resulted in a significant increase in shoot and root dry matter production in the uneroded soil but not in the eroded soil. The dry matter yields of leucaena when grown in association with _G. aggregatum_ or _G. mosseae_ were similar. The values were significantly higher than when leucaena was grown in association with _G. etunicatum_. Dry matter production of leucaena was significantly higher in the uneroded soil than in the eroded soil.

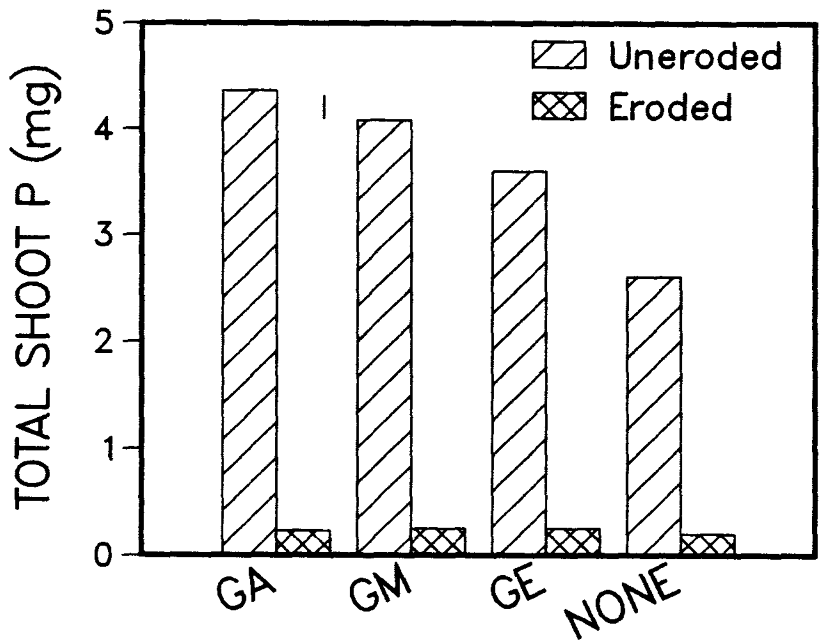

FIG. 4.8. The influence of VAM inoculation on shoot P content of leucaena grown in uneroded or eroded soil. Vertical bar represents LSD at the 5% level. GA = _G._ _aggregatum_, GM = _G._ _mosseae_, GE = _G._ _etunicatum_.

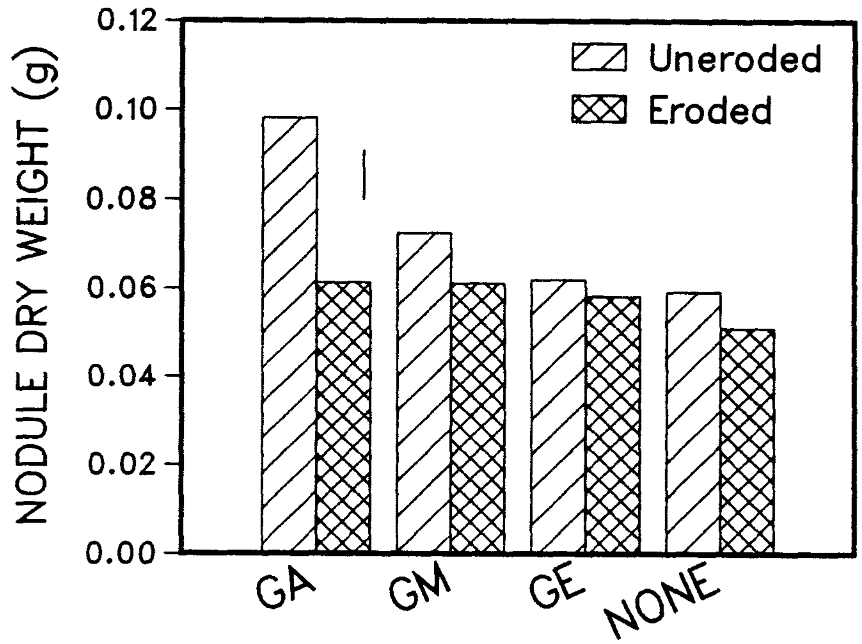

FIG. 4.9. The influence of VAM inoculation on nodule dry matter production of leucaena grown in uneroded or eroded soil. Vertical bar represents LSD at the 5% level. GA = *G. aggregatum*, GM = *G. mosseae*, GE = *G. etunicatum*.

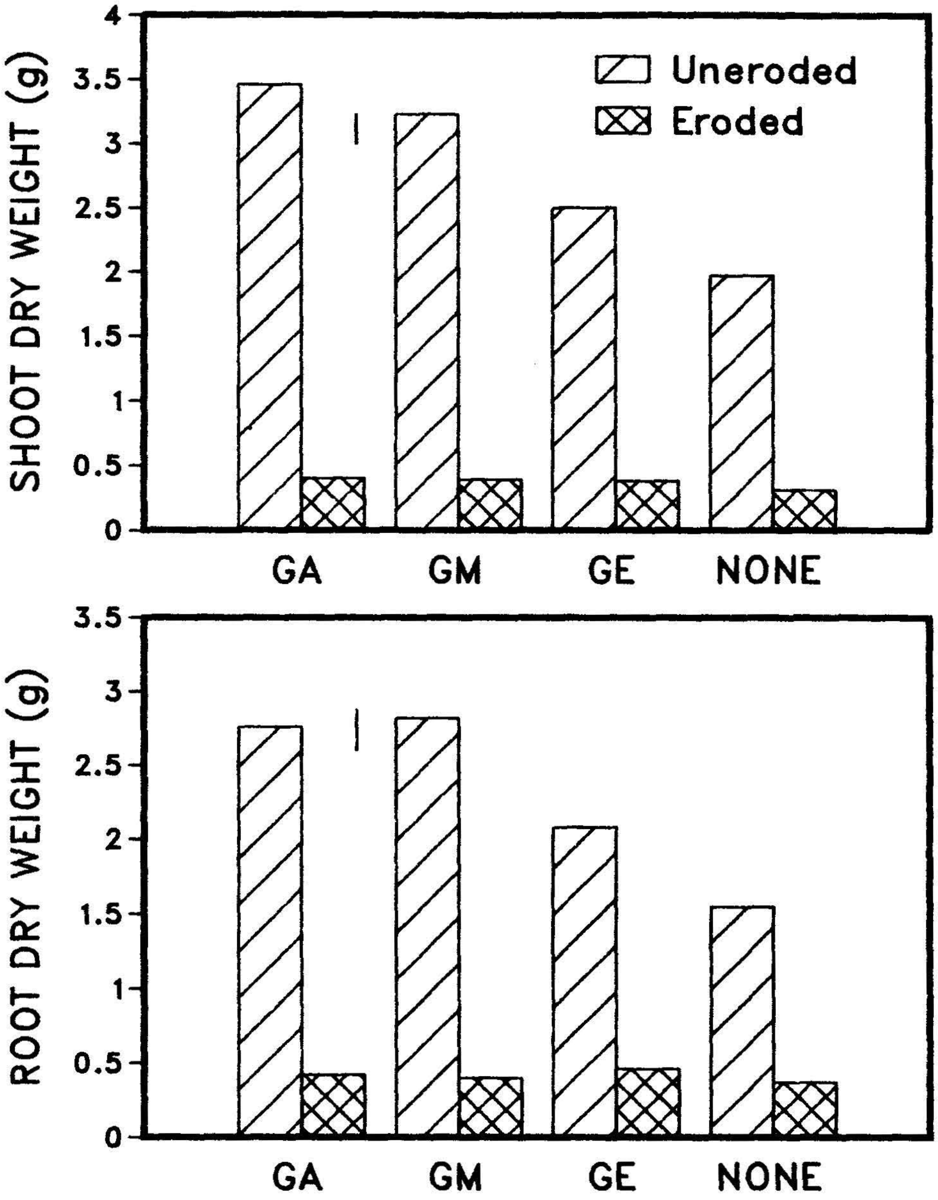

FIG. 4.10. The influence of VAM inoculation on dry matter production of leucaena grown in uneroded or eroded soil. Vertical bars represent LSD at the 5% level.
GA = G. aggregatum, GM = G. mosseae, GE = G. etunicatum.

DISCUSSION

Growth measurements made on cowpea and leucaena indicate a severe supression on their growth in the eroded soil which was not alleviated even when the soil was inoculated with VAM fungus. This indicates that probably low nutrient content of the eroded soil was limiting the performance of the endophytes. This view is supported by the fact that there was a definite growth response to inoculation in the uneroded soil.

The extent of colonization of root by VAM fungi in the eroded soil was not in agreement with that of plant growth. Unlike dry matter production, there was a significant increase in root colonization due to inoculation. This indicates that although plant roots had been infected, they were not effective in stimulating plant growth, i.e., infectivity and effectivity were not correlated. This result was also supported by the effectivity data (P content of leaf discs or subleaflets) which did not change significantly due to VAM inoculation. Plenchette et al. (12) observed a lack of correlation between the percentage of root colonization and stimulation of growth of strawberry grown on calcined montmorillonitic clay. Differences in infectivity and effectivity of VAM species have also been reported by several other workers (1,4,14,15,16,17). The ability to produce rapid and extensive infection is one of the factors contributing to the effectiveness of a VAM fungus (2). Wilson and Trinick (16) hypothesized that one species is more infective than another, probably due to its greater ability to produce infection points.

The reduced infectivity of VAM fungi in the eroded soil compared to that of the uneroded soil could be due to nutritional factors (6,9). The differences observed between the trends of root colonization of cowpea and leucaena by different species of VAM fungi signify preferences exhibited by VAM species towards host plants. Due to suppression of the effectivity of VAM fungi (in terms of plant growth and changes in leaf P status) in the eroded soil, their relative performances could not be evaluated. In uneroded soil, on the other hand, variation in performances was observed. Among the species of VAM fungi tested, _G. aggregatum_ was consistently associated with the highest growth response while _G. etunicatum_ was associated with the least. This shows that one species of VAM fungus is better adapted in the soil environment than another. This is also indicative of the existence of some degree of specificity between endophytes and host plants.

Low nutrient content of the eroded soil, especially P, also reduced the extent of nodulation. This is because of the extra requirement of P needed for nitrogen fixation. The trend of nodulation in the uneroded soil inoculated with different species of VAM fungi was in the same order as that of root colonization or shoot P content. This emphasizes the relationship between nodulation, root colonization and P uptake of leguminous plants.

The results of this study indicate a severe suppression in the effectivity of VAM species in eroded soils.

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CHAPTER 5

OPTIMIZATION OF SOIL SOLUTION PHOSPHORUS FOR MAXIMUM VAM EFFECTIVENESS AND NODULATION OF COWPEA AND LEUCAENA GROWN ON ERODED SOIL

INTRODUCTION

Increased uptake of phosphorus has been determined to be the main reason for improved plant growth associated with the vesicular-arbuscular mycorrhizal (VAM) symbiosis (4,9). VAM fungi, thus, could play an important role in improving plant growth in soils that are either phosphorus deficient or have a high P-fixing capacity. Subsurface soil horizons, which are exposed as a result of topsoil removal by erosion, are not only low in P but also have a high P-sorption capacity (14), and hence, improved plant growth on these soils may depend on the presence of active VAM fungi. The activity and symbiotic effectiveness of VAM fungi is influenced by various soil factors. One of these factors has to do with the level of available soil P. Phosphorus is important because the activity of VAM fungi is known to be adversely influenced by high levels of P and also because there appears to be an optimum P level below which the mycorrhizal activity declines (11,22).

The influence of P on the activity of VAM fungi varies from soil to soil (13). However, this variation is most likely to be a function of the difference in the P adsorbing capacity of the various soils rather than a real measure of the effect of P on VAM fungi (8,14,16). On the other hand, the optimum P level for a mycorrhizal association could vary depending on the VAM endophyte as well as the host species involved (3,5,7,20,22).

Recently, Habte and Manjunath (11) determined the soil solution P level required for maximum effectiveness in the symbiotic interaction involving the VAM fungus Glomus fasciculatum and Leucaena leucocephala. Optimal soil solution P levels for mycorrhizal associations formed by other leguminous plants have not been determined. The objectives of this study were (1) to determine the external P requirement of mycorrhizal cowpea grown in eroded and uneroded soil and (2) to determine the optimum P level for establishing mycorrhizal leucaena and cowpea in non-fumigated eroded and uneroded soil.

In order to fulfill the above mentioned objectives, two experiments were conducted. The experiments were as follow:

Experiment 1: Determination of the external P requirement of mycorrhizal cowpea grown in eroded and uneroded soil.

Experiment 2: Determination of optimum phosphorus level for establishing mycorrhizal cowpea and leucaena in non-fumigated eroded and uneroded soil.

MATERIALS AND METHODS

Experiment 1

Most of the methods and materials used in this experiment were the same as those described in Chapter 2. The portion of methods and materials that were unique to this experiment are described below.

A phosphorus sorption isotherm (6) was used to establish four target levels of phosphorus in the soil solution. The levels were: initial P in the soil solution (0.009 mg/l for the uneroded soil and 0.003 mg/l for the eroded soil), 0.026, 0.046 and 0.087 mg P/l. The pots containing the soils were fumigated in a gas-tight chamber twice at an interval of 10 days by exposing them to 48 g of methyl bromide and 1.0 g of chloropicrin per chamber (volume = 0.712 m^3) for 5 days. After the final fumigation the pots were removed from the chamber and allowed to stand for two weeks to dissipate the fumigants from the soil.

Inoculation of soil with a VAM fungus was achieved by mixing thoroughly 50 g portions of crude inoculum of *Glomus aggregatum* with the soil contained in each pot. Treatments consisted of plants grown on eroded and uneroded soils with 4 target levels of soil solution phosphorus with or without VAM inoculation. The development of VAM activity was monitored by determining the P content of discs of cowpea leaves. Plants were grown for 38 days, after which time measurements of shoot and root dry matter production, nodulation, shoot and root P content and colonization of roots by VAM fungi were made.

Experiment 2

Materials and methods for this experiment were the same as described in Chapter 2 and in Experiment 1 of this chapter. Soil used in this experiment and in all the subsequent experiments were not fumigated.

RESULTS

Experiment 1

The extent of colonization of cowpea roots by *G. aggregatum* increased with increases in the concentration of soil solution P up to 0.046 mg P/l (Fig. 5.1). However, colonization was depressed as the concentration of phosphorus was increased above this level. No evidence of VAM infection was observed in the roots of uninoculated plants.

The changes in leaf disc P content noted when cowpea was grown in eroded and uneroded soil at varying soil solution P levels in the presence or absence of *G. aggregatum* are depicted in Fig. 5.2. When P was not added to the soils, the P content of leaf discs of plants grown in the inoculated uneroded soil increased significantly as a function of time after a lag period of 17 days, attaining a peak value of about 10 g P at 22 days from planting. Phosphorus content in discs of the remaining treatments did not appreciably change with time. Increasing the soil solution P level to 0.026 mg/l increased the P content of leaf discs of inoculated plants grown in both eroded

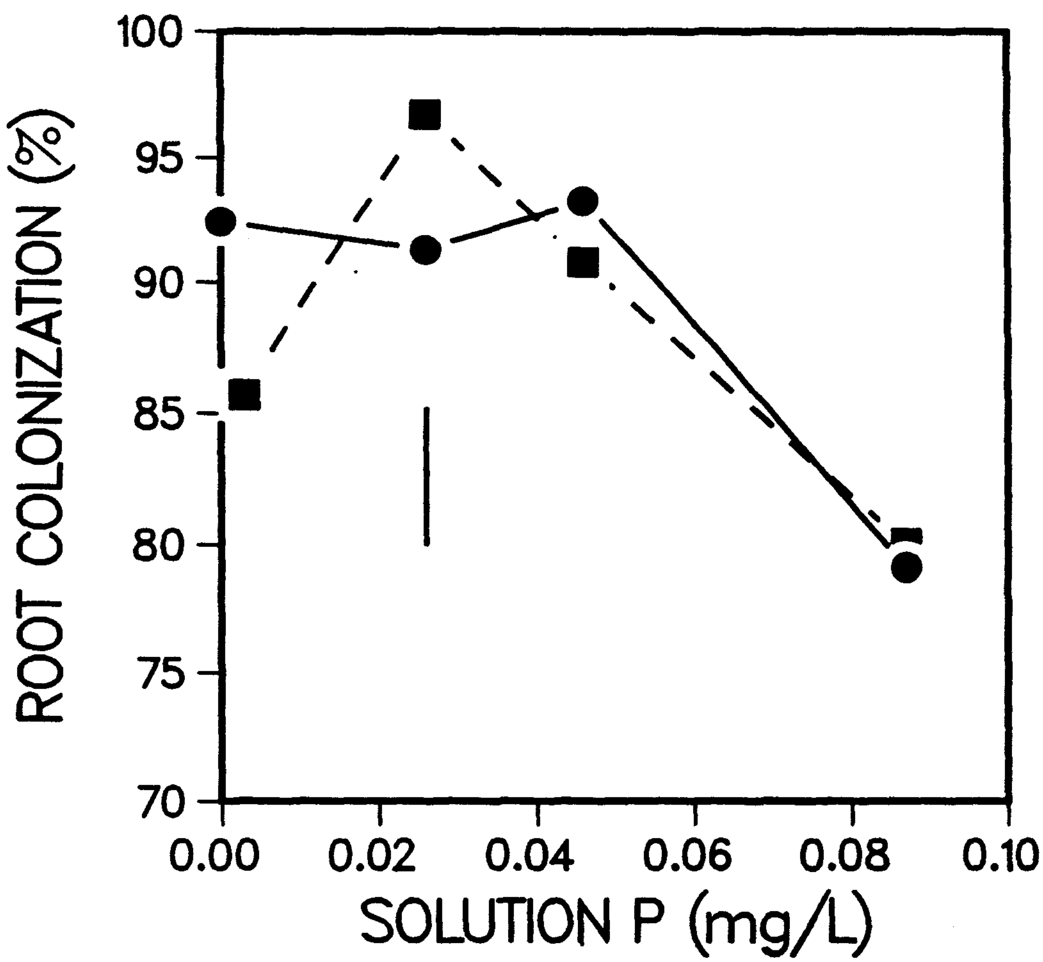

FIG. 5.1. The influence of P and VAM inoculation on the extent of colonization of roots of cowpea grown in uneroded or eroded soil. Vertical bar represents LSD at the 5% level. (●) = uneroded, inoculated; (■) = eroded, inoculated.

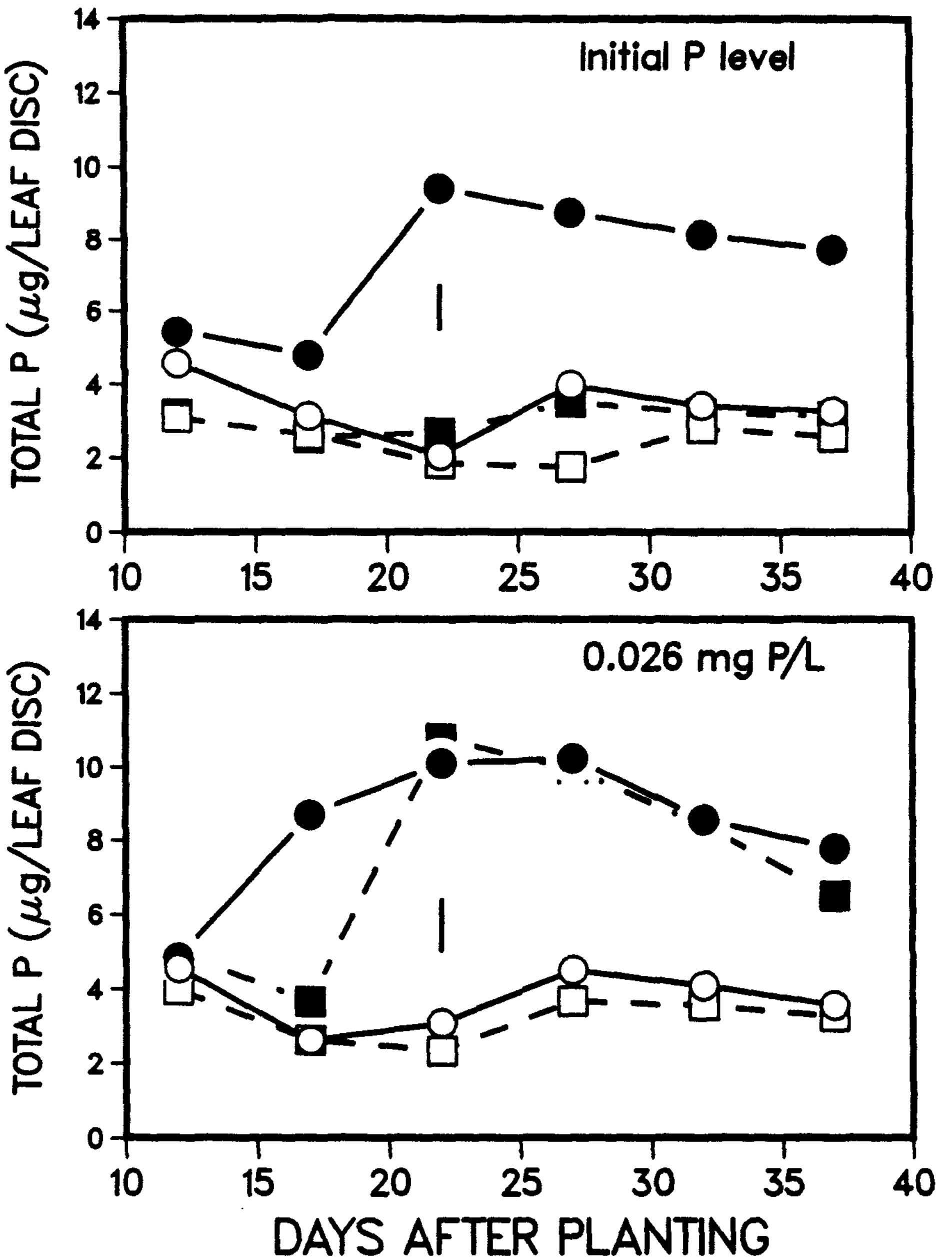

FIG. 5.2. The influence of P and VAM inoculation on the development of mycorrhizal effectiveness in cowpea grown in uneroded or eroded soil. Vertical bars represent LSD at the 5% level. (○) = uneroded, uninoculated; (●) = uneroded, inoculated; (□) = eroded, uninoculated; (■) = eroded, inoculated.

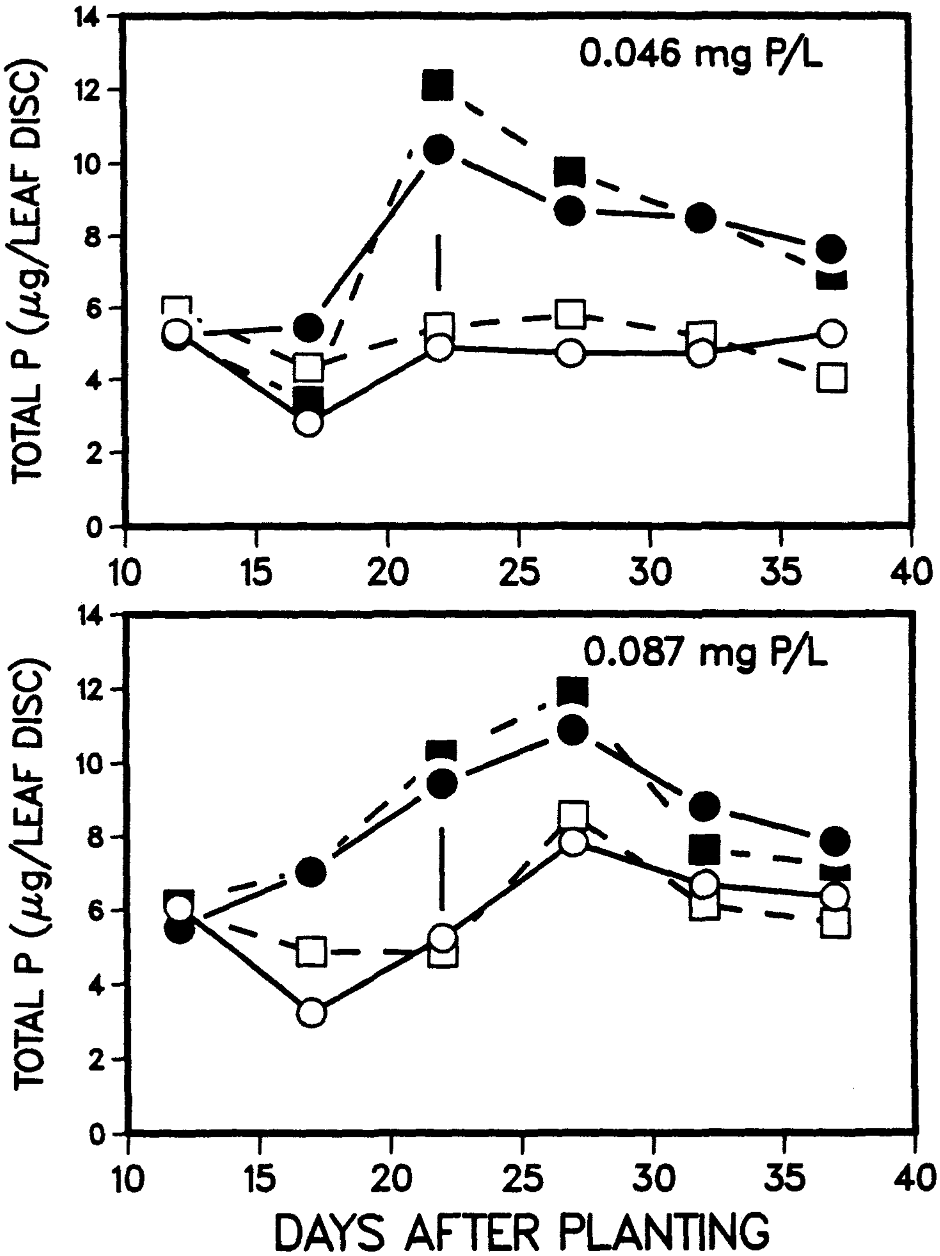

FIG. 5.2. Continuation.

and uneroded soils. Peak mycorrhizal activity measured in terms of P content of leaf discs was observed at 22-27 days from planting. Vesicular-arbuscular mycorrhizal activity in the uneroded soil was initiated much earlier than in the soil not fertilized with P. Mycorrhizal cowpea grown in the eroded and uneroded Wahiawa soil had peak P content values that were not significantly different from each other eventhough there was a 17-day delay before VAM activity was detected in the eroded soil. This peak was followed by a decline in P content. A further increase in the P concentration of the soil solution did not significantly alter the pattern of P accumulation in mycorrhizal cowpea grown in the eroded and uneroded Wahiawa soil. It did, however, increase the P content of leaf discs of cowpea grown in the uninoculated eroded and uneroded soil. Leaf discs of the latter plants had similar P contents, but they were significantly lower than those observed in plants grown in eroded and uneroded soils in the presence of G. aggregatum. Increasing the soil solution P concentration seemed to induce a lag in the VAM activity of plants grown in the eroded and uneroded soil. At P concentrations above 0.046 mg/l, P contents of discs of plants grown in both eroded and uneroded soils inoculated with G. aggregatum increased rapidly (i.e. no lag). The overall pattern of mycorrhizal activity measured in terms of P content of cowpea leaf discs did not differ from that observed when activity was monitored in terms of P concentration of leaf discs [Fig. B.2 (Appendix B)].

Shoot P concentration and shoot P content of cowpea as affected by P application are illustrated in (Fig. 5.3). Mycorrhizal cowpea

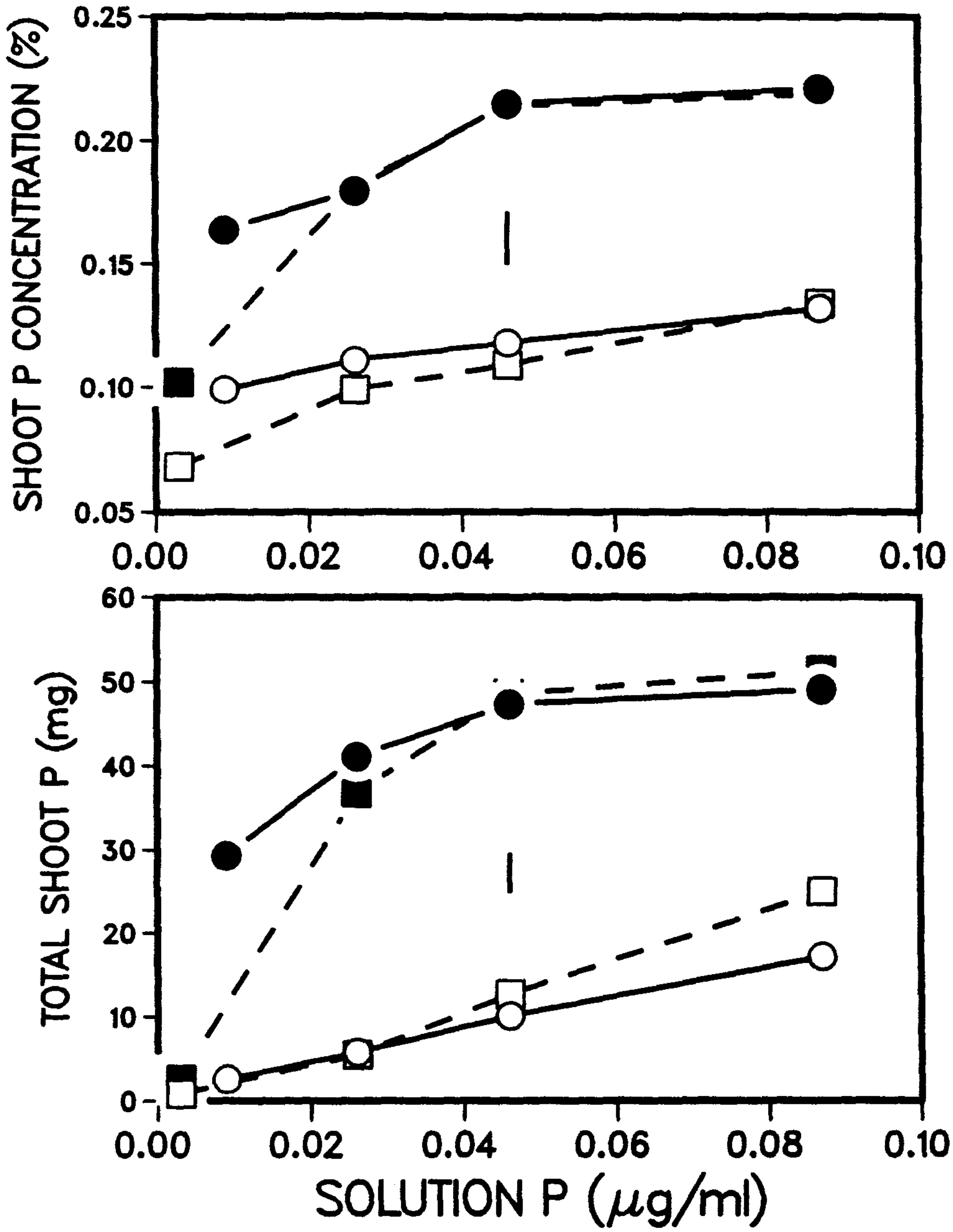

FIG. 5.3. The influence of P and VAM inoculation on shoot P status of cowpea grown in uneroded or eroded soil. Vertical bars represent LSD at the 5% level. (○) = uneroded, uninoculated; (●) = uneroded, inoculated; (□) = eroded, uninoculated; (■) = eroded, inoculated.

had significantly higher levels of shoot P than nonmycorrhizal ones at all levels of soil P except at the lowest level. In the absence of added P, the P status of leaf discs was higher in the uneroded inoculated soil than in the eroded inoculated soil. This difference was removed when P was added at 0.026 mg/l. Phosphorus concentration of leaf discs was linearly related to P concentration of shoots ($r = 0.87^{**}$) (Fig. 5.4).

Nodule dry weight increased linearly with increase in P in the uninoculated soil irrespective of erosion treatment (Fig. 5.5). Nodule dry weight also linearly increased with increase in the soil solution P in the inoculated soil but stabilized when P in the soil solution was increased beyond 0.046 mg/l, and mycorrhizal plants produced similar nodule dry weight. At all levels of soil solution P except the initial level, the mycorrhizal plants had significantly higher quantities of nodules than the non-mycorrhizal ones.

The influence of soil solution P and mycorrhizal inoculation on dry matter accumulation of cowpea are depicted in Fig. 5.6. When P was not added to the soil samples, there was no response to mycorrhizal inoculation except in the uneroded soil where inoculation resulted in shoot weight that was 8 times greater than that of other treatments. By raising soil solution P level to 0.026 mg/l, the shoot dry weight of inoculated plants was increased by 28% in the uneroded soil and by 8-fold in the eroded soil. Further increases in the level of soil solution P did not increase the shoot dry weight of inoculated plants appreciably. Uninoculated plants, on the other hand, responded to all levels of P applied. Shoot dry weight of inoculated plants was

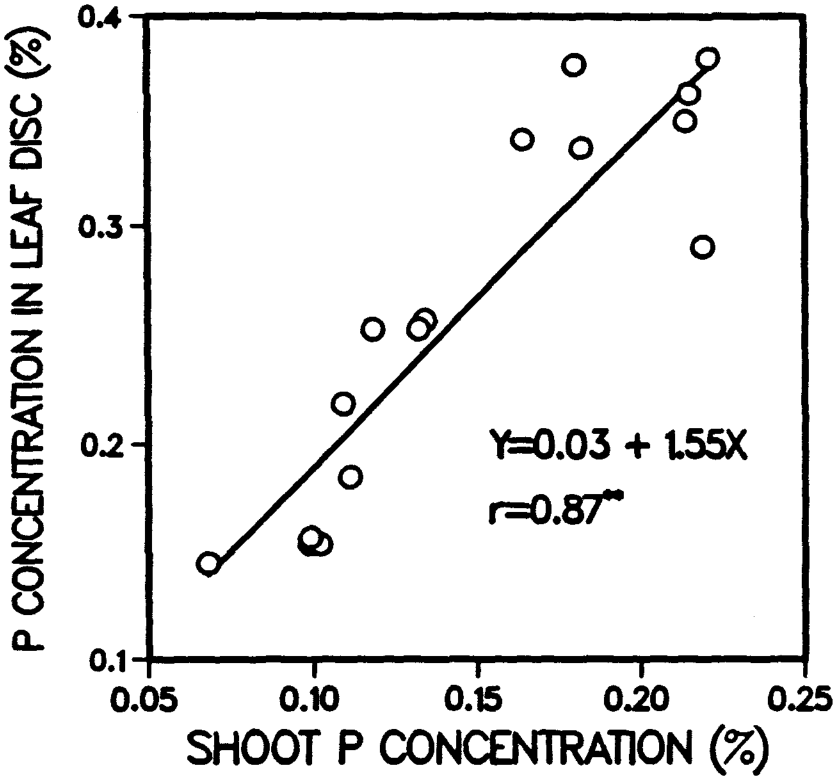

FIG. 5.4. Relationship between leaf disc and shoot P concentration of cowpea.

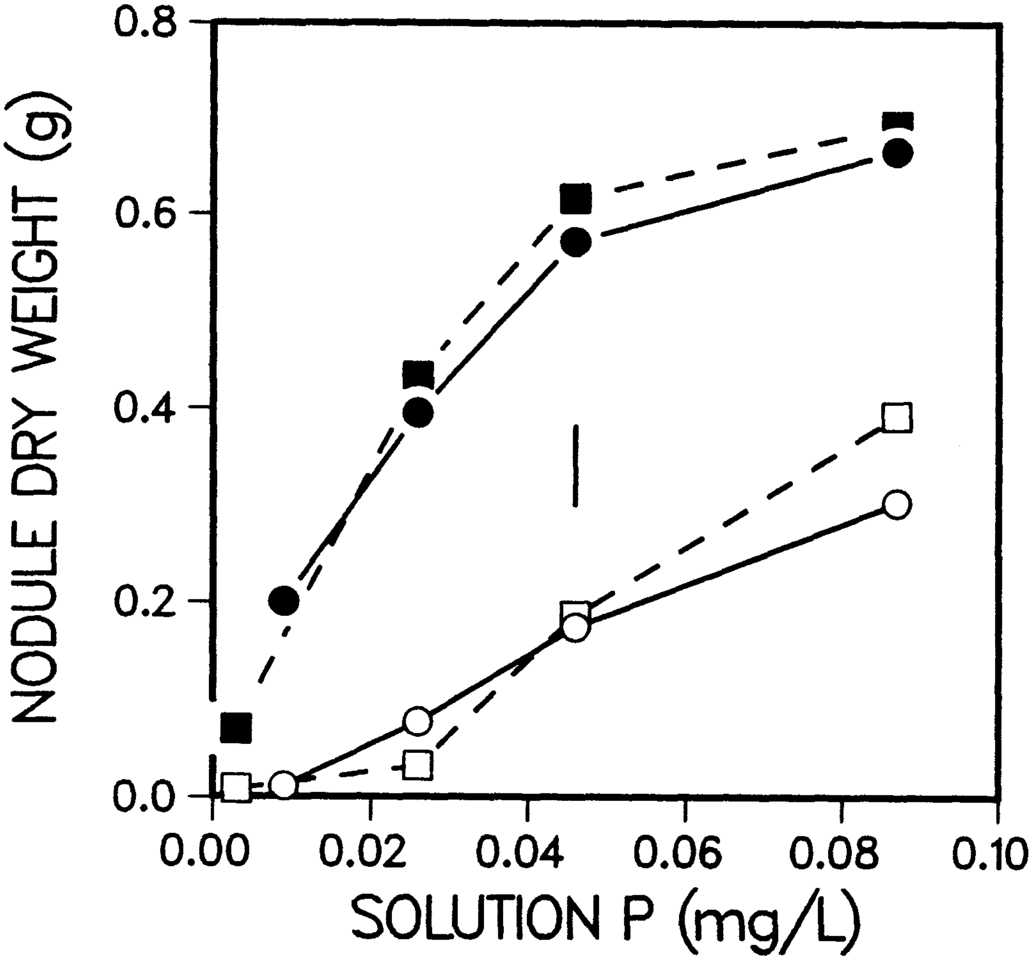

FIG. 5.5. The influence of P and VAM inoculation on nodule dry matter production of cowpea grown in uneroded or eroded soil. Vertical bar represents LSD at the 5% level. (○) = uneroded, uninoculated; (●) = uneroded, inoculated; (□) = eroded, uninoculated; (■) = eroded, inoculated.

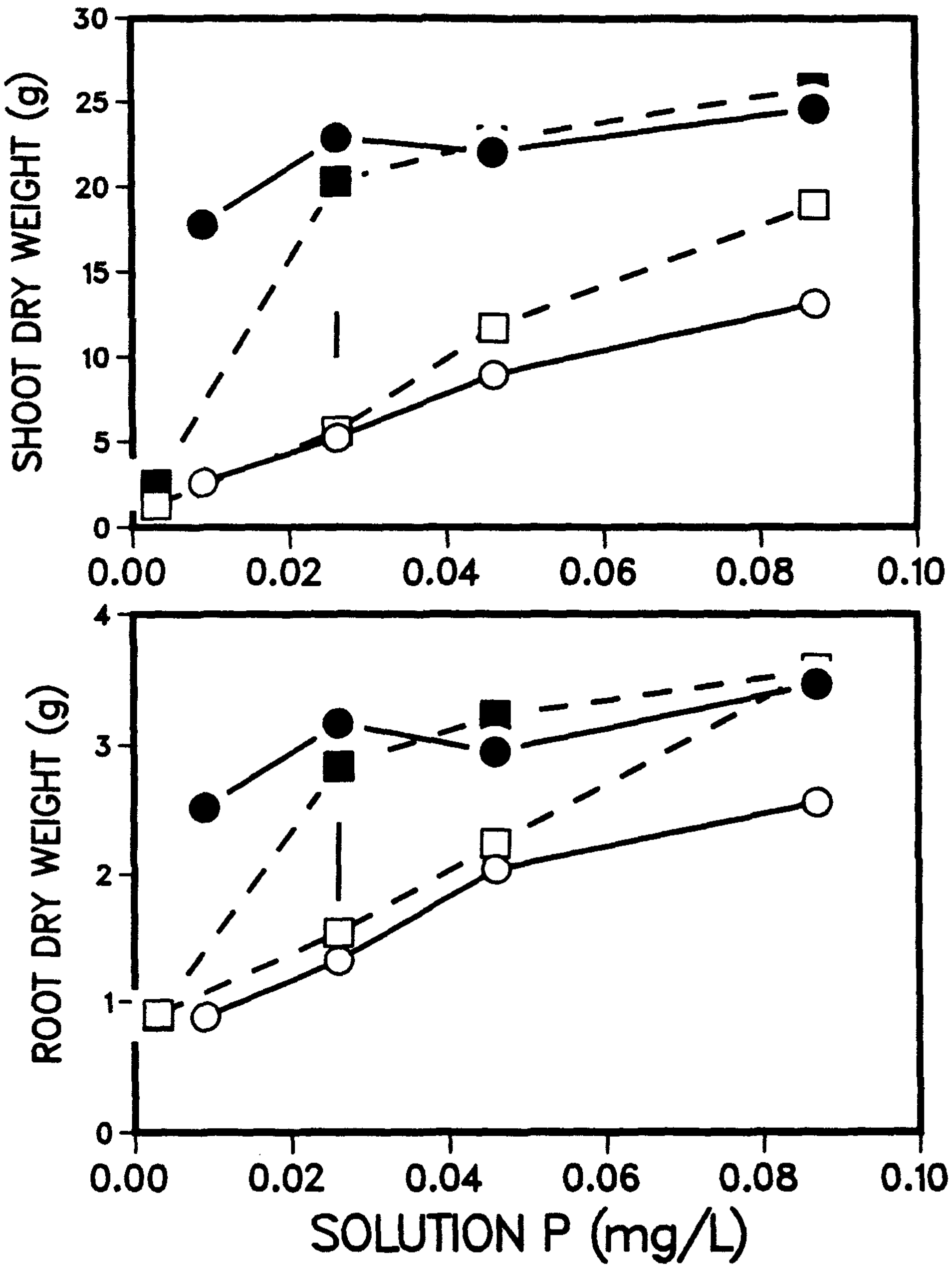

FIG. 5.6. The influence of P and VAM inoculation on dry matter production of cowpea grown in uneroded or eroded soil. Vertical bars represent LSD at the 5% level. (○) = uneroded, uninoculated; (●) = uneroded, inoculated; (□) = eroded, uninoculated; (■) = eroded, inoculated.

always significantly greater than that of uninoculated plants except at the initial soil solution P level where cowpea did not respond to the inoculation of eroded soil. Maximum mycorrhizal effect in terms of shoot growth was at 0.026 mg P/l in soil solution in both eroded and uneroded soils. The shoot dry weights of mycorrhizal plants grown at the soil solution P level of 0.026 mg/l were 74 and 7 percent higher in uneroded and eroded soils, respectively, compared to the shoot dry weights of unaided plants grown at the highest soil solution P level tested (0.087 mg/l). Root dry weight was significantly increased by inoculation at the soil solution P levels of 0.026 and 0.046 mg/l. At the highest level of P tested, inoculation did not seem to have effect in the eroded soil. The trend of root growth was, by and large, similar to that of shoot growth.

Figure 5.7 shows the influence of mycorrhizal inoculation on root/shoot ratio at different levels of soil solution P. At the initial P level, root/shoot ratio was maximum (0.71) for inoculated plants grown on eroded soil and minimum for inoculated plants grown in uneroded soil. As the soil solution P level was raised to 0.026 mg/l, the root/shoot ratio decreased significantly in all cases except in mycorrhizal plants grown on the uneroded soil. This soil had the lowest root/shoot ratio which was maintained across the P levels. Root/shoot ratio appeared to stabilize at P levels higher than 0.026 mg/l.

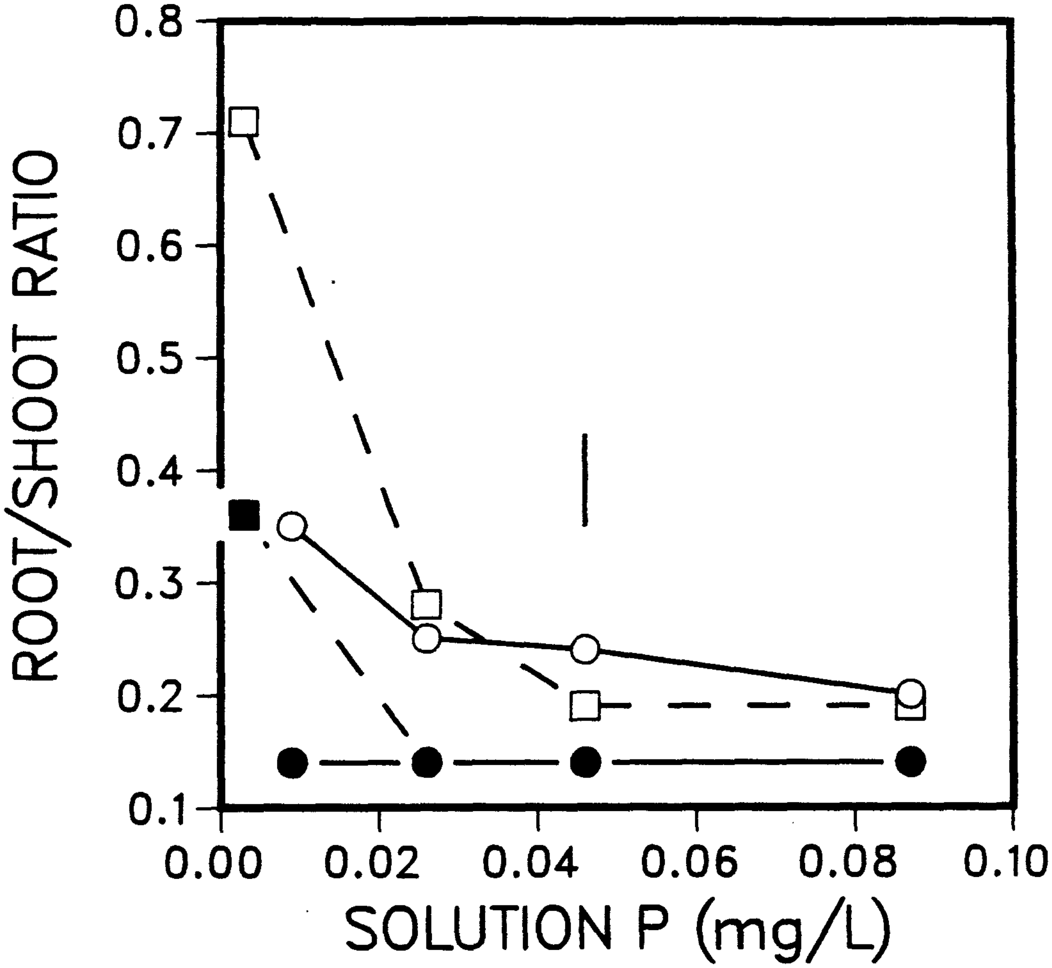

FIG. 5.7. The influence of P and VAM inoculation on root/shoot ratio of cowpea grown in uneroded or eroded soil. Vertical bar represents LSD at the 5% level. (○) = uneroded, uninoculated; (●) = uneroded, inoculated; (□) = eroded, uninoculated; (■) = eroded, inoculated.

Experiment 2

Cowpea. The extent of colonization of cowpea roots by VAM fungi in the eroded soil increased with increasing levels of soil solution P reaching maximum at 0.026 mg P/l and then decreased at the highest level (Fig. 5.8). The extent of colonization of cowpea roots in the uneroded soil did not change significantly with increasing levels of soil solution P. Mycorrhizal colonization of roots was always higher in the eroded than in the uneroded soil but the difference was significant only at the soil solution P level of 0.026 mg/l.

Mycorrhizal activity monitored by determining the P content of leaf discs at different soil solution P levels is illustrated in Fig. 5.9. When P was not added to the eroded soil, the initial mycorrhizal activity was significantly depressed compared to when the soil was fertilized with P. The activity did not change appreciably with time. In the uneroded unamended soil, on the other hand, mycorrhizal activity increased significantly at 17 days after planting (DAP) and peaked at 22 DAP, after which time the activity started declining. When P was added to the soil samples, mycorrhizal activity was initiated with a lag period except at the P level of 0.026 mg/l in the eroded soil and 0.046 mg/l in the uneroded soil. The peak mycorrhizal activity was attained at 22 days from planting after which time the activity declined. The mycorrhizal activity did not differ significantly when the soil samples were amended with different levels of soil solution P. Similar results were obtained when mycorrhizal activity was monitored in terms of the P concentration of leaf discs [Fig. B.3 (Appendix B)].

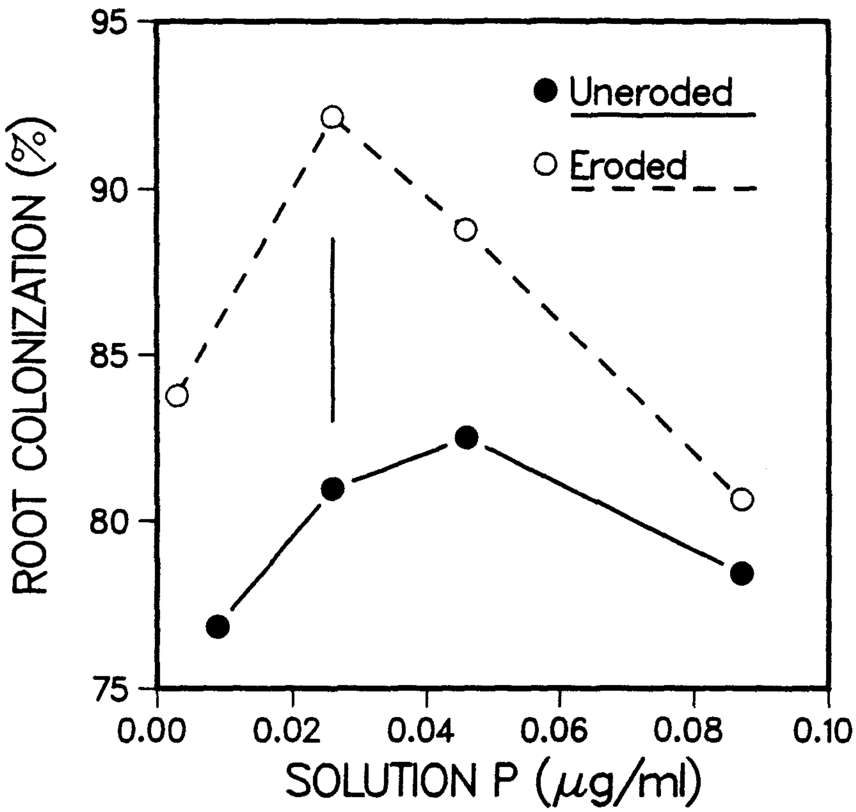

FIG. 5.8. The influence of P on the extent of VAM colonization of roots of cowpea grown in uneroded or eroded soil inoculated with G. aggregatum. Vertical bar represents LSD at the 5% level.

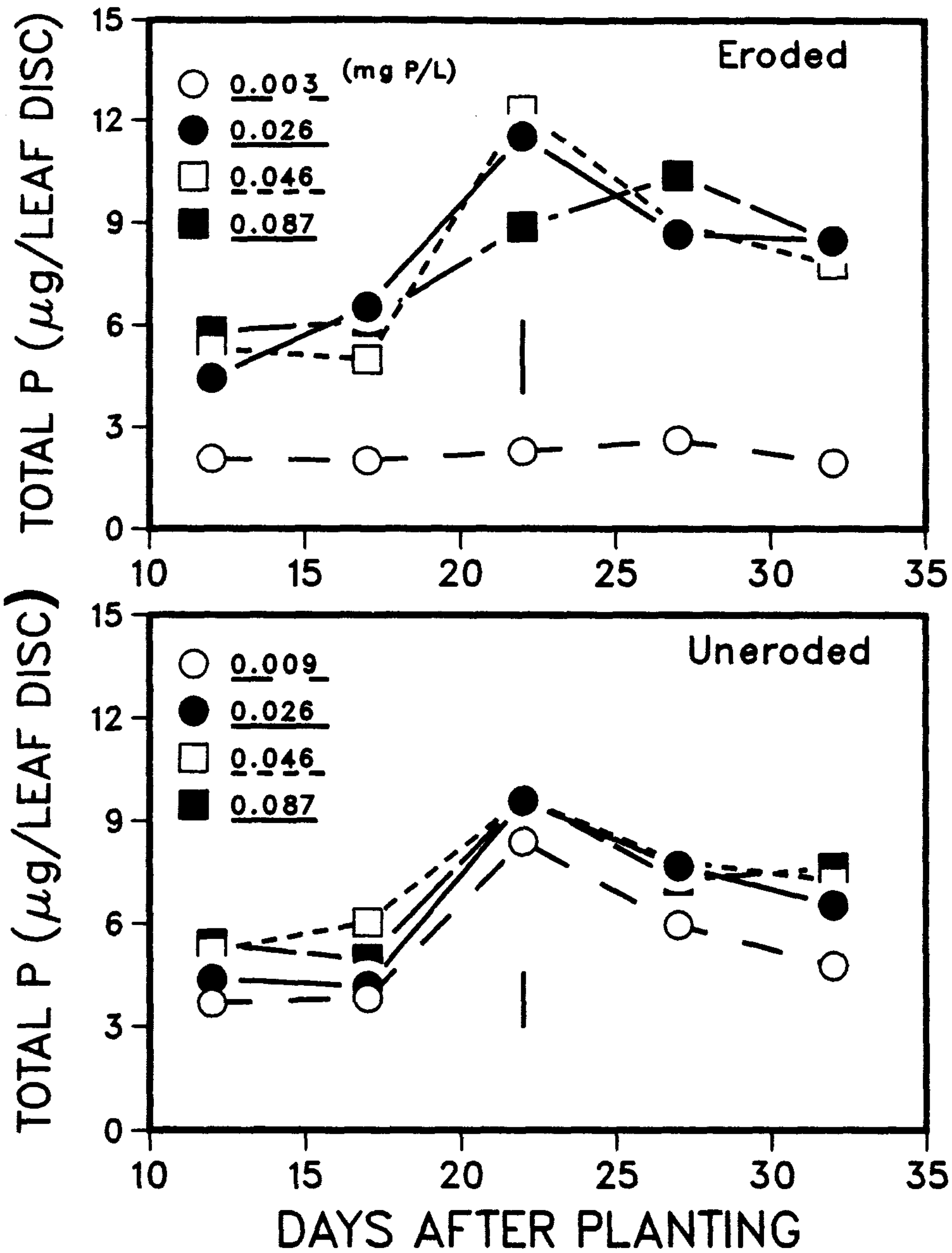

FIG. 5.9. The influence of P on the development of mycorrhizal effectiveness in cowpea grown in eroded or uneroded soil inoculated with G. aggregatum. Vertical bars represent LSD at the 5% level.

Shoot P concentration and total shoot P content of cowpea were significantly lower in the eroded soil than in the uneroded soil when P was not added (Fig. 5.10). With addition of P, there was an increase in the shoot P status upto a soil solution P level of 0.046 mg/l. The shoot P status of cowpea did not increase further above 0.046 mg P/l.

Nodule dry matter production increased significantly with increase in P upto a soil solution level of 0.046 mg/l (Fig 5.11). Nodule dry weight was similar in the eroded and uneroded soils at all the soil solution P levels except at the initial P level.

The influence of soil solution P on dry matter production of cowpea inoculated with G. aggregatum is depicted in Fig. 5.12. In the absence of added P, the shoot dry weight of cowpea grown in the eroded soil was severely suppressed compared to those grown in the uneroded soil. When the soil solution P level was raised to 0.026 mg/l, shoot dry matter production of cowpea increased by 46% in the uneroded soil and by 900% in the eroded soil, thus eliminating the initial suppression in growth that was observed in the eroded soil in the absence of added P. With increase of soil solution P level upto 0.046 mg/l, the shoot dry weights of cowpea also increased further. There was, however, no increase in shoot dry weight above this P level. Root dry matter production was similar in trend to that of shoot dry matter production except that the root dry weight did not increase significantly above the soil solution P level of 0.026 mg/l.

Figure 5.13 shows the influence of soil solution P levels on root/shoot ratio of cowpea. At the initial P level, the root/shoot

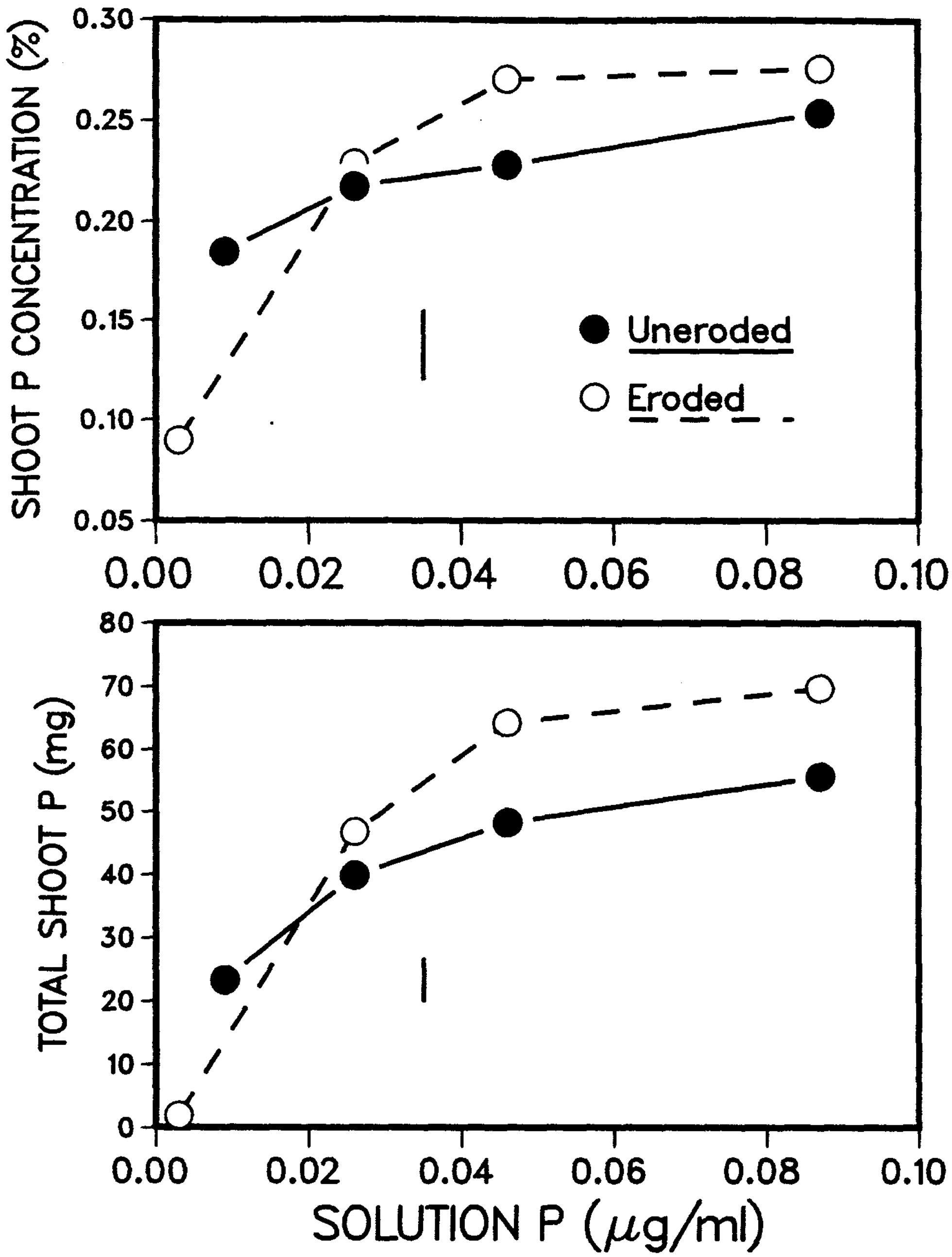

FIG. 5.10. The influence of P on shoot P status of cowpea grown in uneroded or eroded soil inoculated with G. aggregatum. Vertical bars represent LSD at the 5% level.

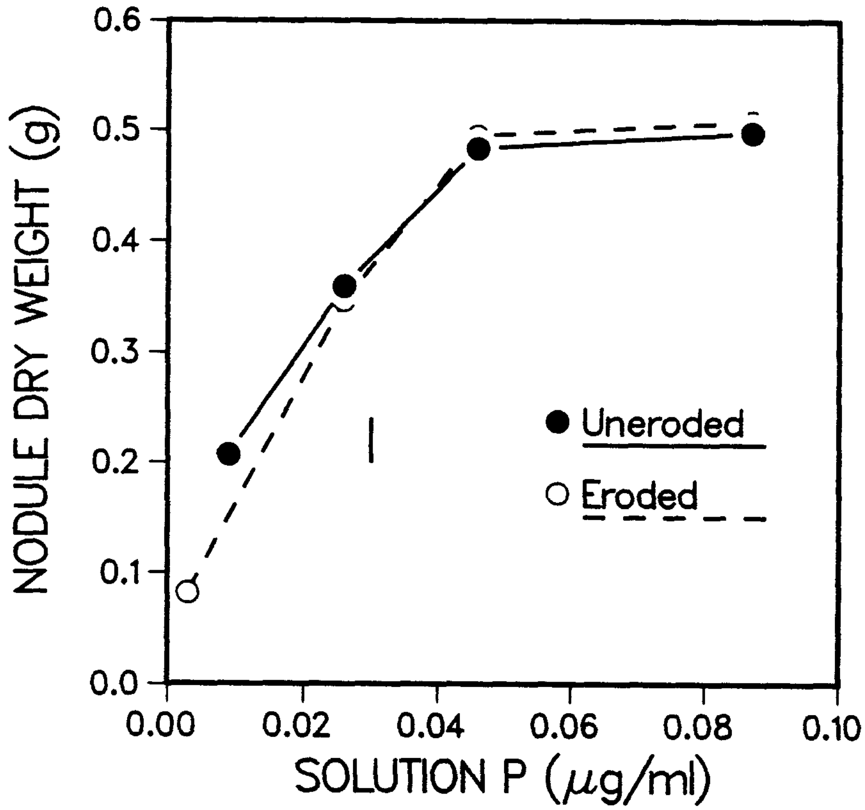

FIG. 5.11. The influence of P on nodule dry matter production of cowpea grown in uneroded or eroded soil inoculated with *G. aggregatum*. Vertical bar represents LSD at the 5% level.

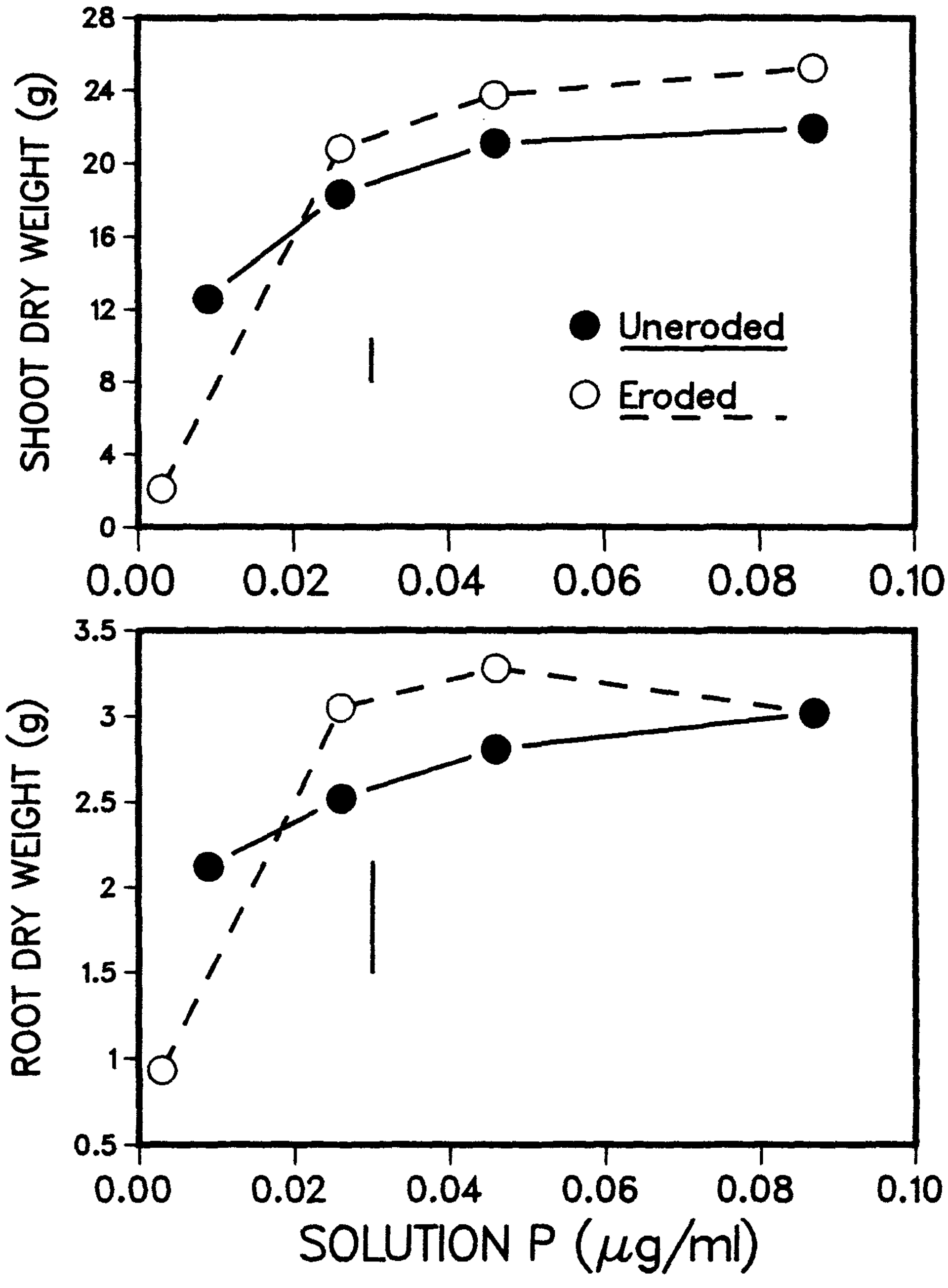

FIG. 5.12. The influence of P on dry matter production of cowpea grown in uneroded or eroded soil inoculated with G. aggregatum. Vertical bars represent LSD at the 5% level.

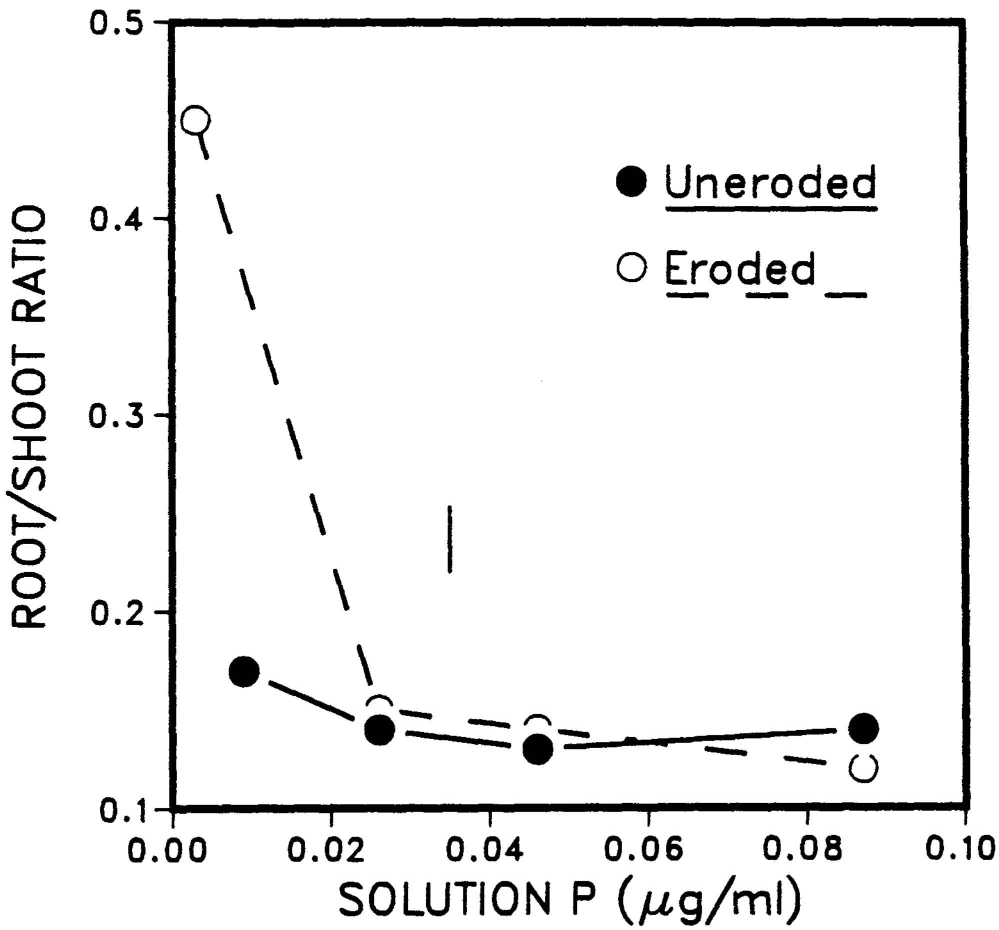

FIG. 5.13. The influence of P on root/shoot ratio of cowpea grown in uneroded or eroded soil inoculated with G. aggregatum. Vertical bar represents LSD at the 5% level.

ratio of cowpea grown in the eroded soil was significantly higher than those observed in the uneroded soil. By adding P upto 0.026 mg/l, the root/shoot ratio of cowpea grown in both the soil samples was reduced to about 0.14. Above 0.026 mg P/l, there was no appreciable change in root/shoot ratio.

Leucaena. The extent of colonization of leucaena roots by VAM fungi increased with increasing levels of soil solution P reaching maximum at 0.026 mg P/l soil solution and then decreased at the highest level (not significantly) (Fig. 5.14). The level of infection was consistently but not significantly higher in the eroded soil than in the uneroded soil and the values ranged from 65 to 85 percent.

Mycorrhizal activity monitored by determining the P content of subleaflets of leucaena at different soil solution P levels is illustrated in Fig. 5.15. When P was not added to the eroded soil, mycorrhizal activity decreased until 17 DAP and then increased slowly, reaching a peak value of about 2 g P at 32 DAP. Mycorrhizal activity at this P level was always lower than at the higher P levels except at 12 DAP. In the uneroded soil, mycorrhizal activity increased begining 12 DAP and reached a peak value of about 8 g P at 27 DAP, after which time the activity started declining and reached a value of 2.5 g P at 37 DAP. When P was added to soil, mycorrhizal activity was initiated without a lag period in the eroded soil and with a lag period in the uneroded soil except at the P level of 0.026 mg/l. Maximum mycorrhizal activity was observed at 22-27 DAP after which time the activity appeared to stabilize. In both the eroded and uneroded soils, mycorrhizal activity was maximum at the soil solution P level

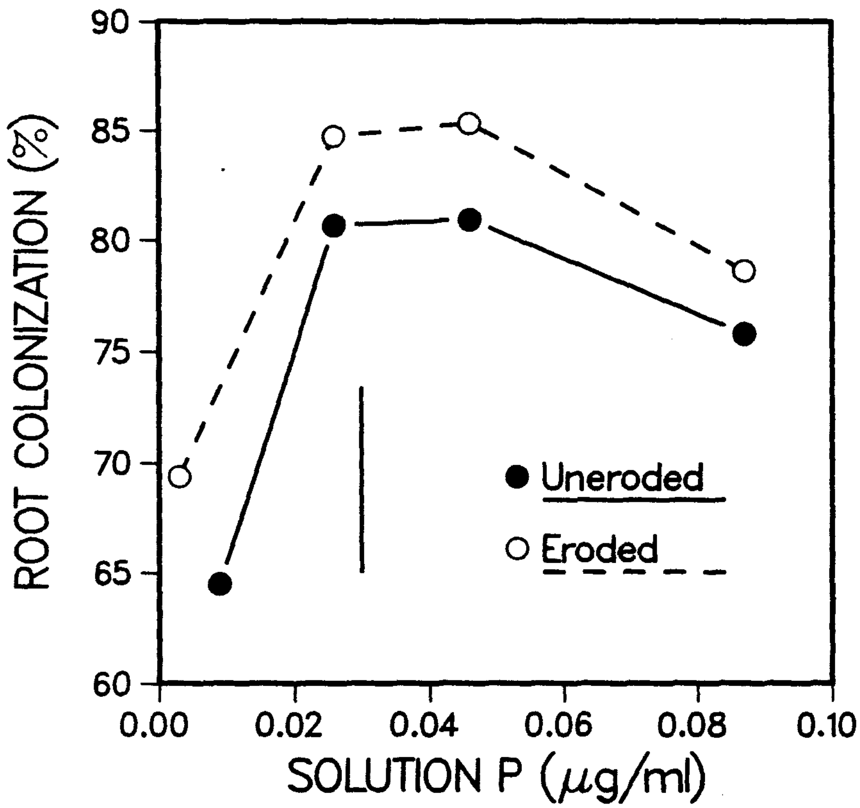

FIG. 5.14. The influence of P on the extent of VAM colonization of roots of leucaena grown in uneroded or eroded soil inoculated with G. aggregatum. Vertical bar represents LSD at the 5% level.

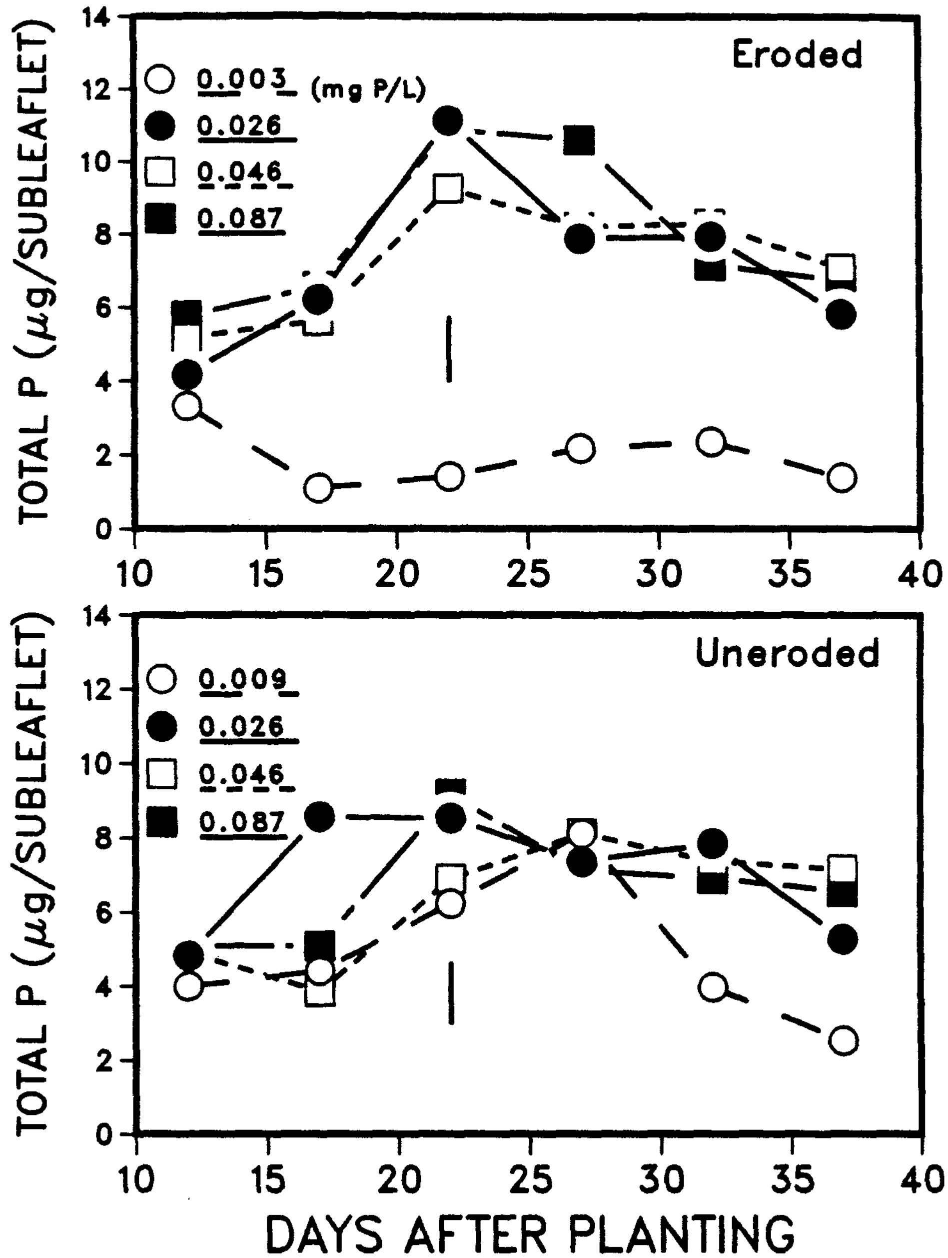

FIG. 5.15. The influence of P on the development of mycorrhizal effectiveness in leucaena grown in eroded or uneroded soil inoculated with *G. aggregatum*. Vertical bars represent LSD at the 5% level.

of 0.026 mg/l. Similar trends were observed when mycorrhizal activity was monitored in terms of the P concentration of subleaflets [Fig. C.2 (Appendix C)].

Shoot P status of leucaena was higher in the uneroderd soil than in the eroded soil in the absence of added P (Fig. 5.16). When the soil solution P level was raised to 0.026 mg/l, there was a significant increase in shoot P concentration and shoot P content of leucaena. There was no further increase in shoot P status at higher soil solution P levels. Nodule dry weight increased significantly with P application in the uneroded soil but not in the eroded soil (Fig. 5.17).

At the initial soil solution P level, the shoot and root dry matter production of leucaena was 1.80 and 1.85 times higher, respectively, in the uneroded soil than that in the eroded soil (Fig. 5.18). When the soil solution P level was raised to 0.026 mg/l, shoot weight increased by 573 percent in the eroded and by 143 percent in the uneroded soil. At higher P levels there was no further increase in shoot dry weight. Root dry matter production was similar in trend to that of shoot dry matter production except that the root dry weight observed at the soil solution P level of 0.087 mg/l was significantly higher in the eroded soil than in the uneroded soil.

Figure 5.19 shows the influence of soil solution P levels on the root/shoot ratio of leucaena. When P was added to get a soil solution P level of 0.026 mg/l, the root/shoot ratio of plants decreased significantly in both the eroded and uneroded soils. At higher P

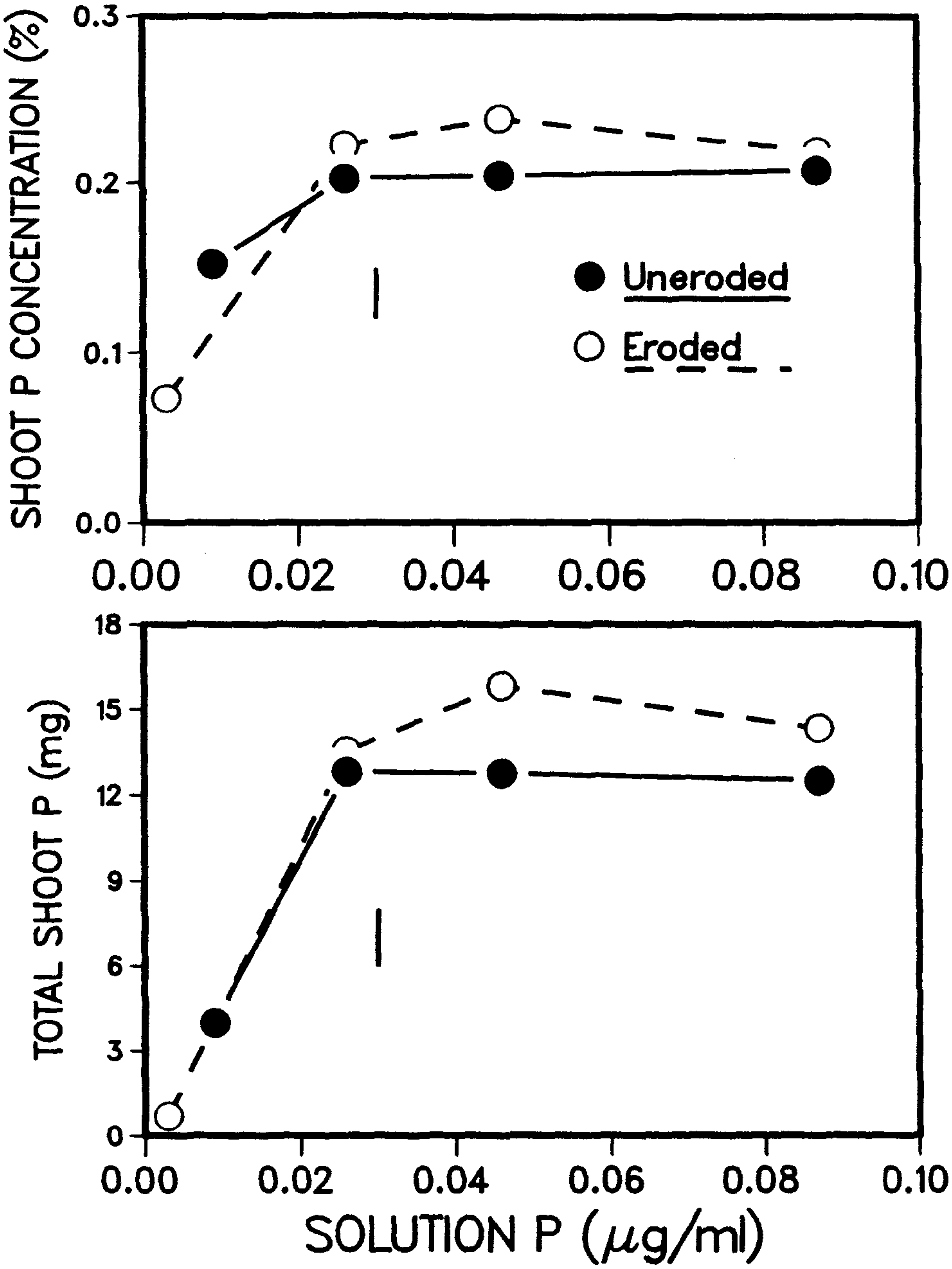

FIG. 5.16. The influence of P on shoot P status of leucaena grown in uneroded or eroded soil inoculated with G. aggregatum. Vertical bars represent LSD at the 5% level.

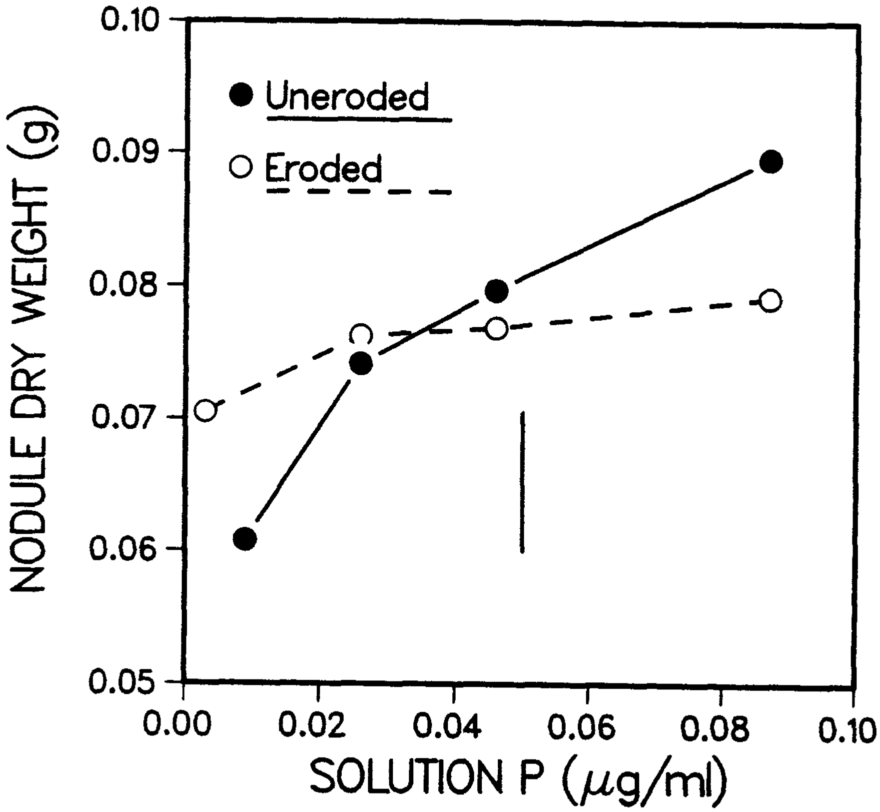

FIG. 5.17. The influence of P on nodule dry matter production of leucaena grown in uneroded or eroded soil inoculated with G. aggregatum. Vertical bar represents LSD at the 5% level.

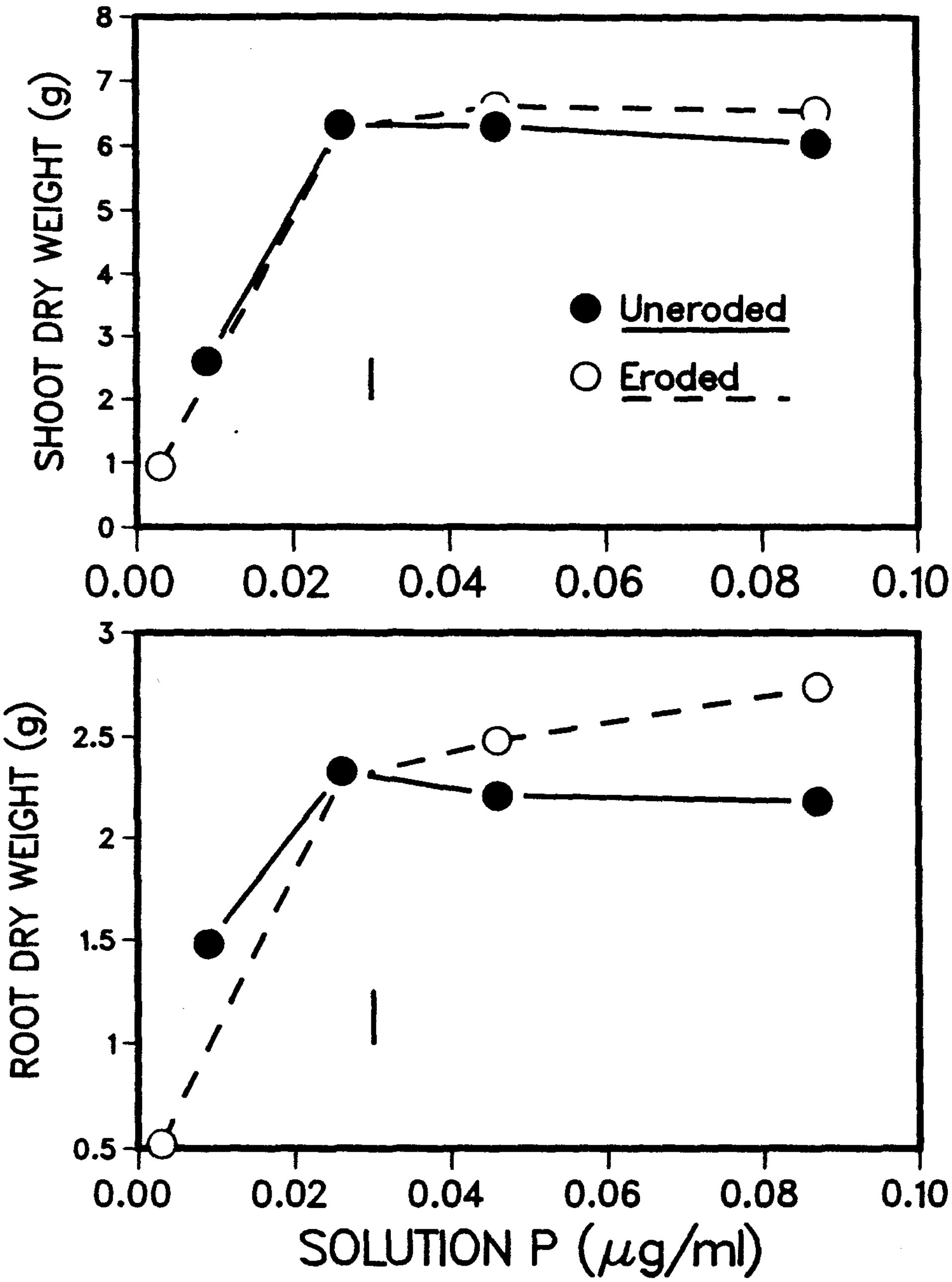

FIG. 5.18. The influence of P on dry matter production of leucaena grown in uneroded or eroded soil inoculated with _G. aggregatum_. Vertical bars represent LSD at the 5% level.

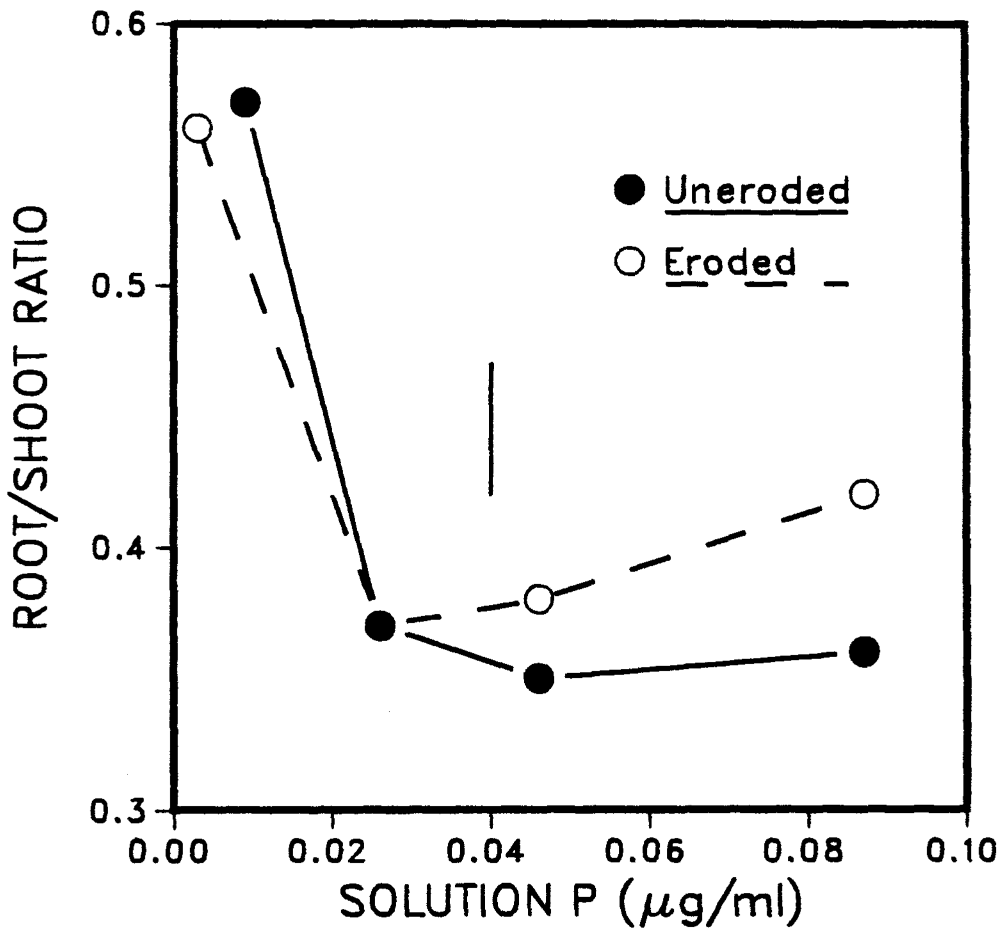

FIG. 5.19. The influence of P on root/shoot ratio of leucaena grown in uneroded or eroded soil inoculated with G. aggregatum. Vertical bar represents LSD at the 5% level.

levels, the root/shoot ratio remained the same in the uneroded soil while it increased in the eroded soil.

DISCUSSION

Experiment 1

The absence of a reliable non-destructive procedure to monitor the development of VAM activity has long been felt. The technique developed recently by Habte et al. (in press), can be used to determine VAM activity in plants that have compound leaves. The results of the present study show that periodical removal of leaf discs and estimating their P content could be used to monitor VAM activity of plants that do not have compound leaf system. A good correlation (r = 0.87) was observed between the P content of leaf discs and shoot P content. Since shoot P content is a standard procedure for assessing VAM effectiveness, P content of leaf discs can be used as a simple non-destructive method for monitoring VAM effectiveness. This procedure revealed that VAM activity in cowpea was initiated as early as 12-17 days from planting while peak activities were noted 22-27 days from planting, depending on the initial P status of the soil and on whether or not the soil was subjected to simulated erosion. The lack of stimulation in P uptake of cowpea due to VAM inoculation of the eroded soil not fertilized with P is indicative of the fact that the level of P initially contained in the soil, i.e., 0.003 mg/l was below the threshold level required for mycorrhizal activity. This view is supported by the fact that there was a detectable level of activity in the uneroded soil which had a higher level of P, i.e., 0.009 mg/l. Furthermore, the leaf P status of plants grown in the inoculated eroded soil increased significantly and reached the same level of P as that attained by plants grown in the uneroded soil when the soil solution P level was increased to 0.026 mg/l.

High levels of P in soil are known to affect VAM infection adversely (12,13,15,17,22). The present investigation revealed that when the soil solution P level was maintained at 0.026 mg/l, the mycorrhizal infectivity and mycorrhizal activity determined in terms of P status of leaf discs were maximum irrespective of soil treatment. This soil solution P level can, thus, be defined as optimum for the symbiotic interaction between cowpea and G. aggregatum. Similar results were reported by Habte and Manjunath (11) who observed a soil solution P level of 0.021 mg/l to be optimum for the symbiotic association between Leucaena leucocephala and G. fasciculatum, currently renamed as G. aggregatum. Despite differences in host species and soil erosion treatment, these values were similar which suggest that soil solution P is a valuable tool for predicting the response of host plants to VAM inoculation. These observations also support the fact that in the presence of VAM fungi, plant species can have similar external P requirements despite differences in their dependence on mycorrhizal fungi. This result supports the observation that mycorrhizal fungi tend to lower the external P requirements of their hosts (5).

The length of time taken for the initiation of symbiotic activity by the endophyte is one way that may be used to evaluate the influence of P on VAM activity. The 17-day lag period observed prior to the initiation of VAM activity in the uneroded unamended soil was reduced by at least 5 days when the initial soil solution P level was adjusted to 0.026 mg/l. When the P level was increased to 0.046 mg/l, the longer lag period observed is probably due to the sensitivity of the endophyte to high P level. The lag in mycorrhizal activity (17 day) observed in the eroded soil even though the soil solution P was at an optimum level (0.026 mg/l), suggests that factors other than P were limiting the initiation of mycorrhizal activity in this soil.

The external P requirement of mycorrhizal cowpea was satisfied at the soil solution P level of 0.026 mg/l. This is indicated from the fact that shoot and root dry weights of inoculated plants did not increase significantly above that level. The external P requirement of the uninoculated plants, on the other hand, was higher than the highest soil solution P level tested, i.e., 0.087 mg/l. This statement is based on the fact that shoot and root dry weights of non-mycorrhizal plants continued to increase with increasing levels of P. It can be seen from these results that nonmycorrhizal cowpea has external P requirement that is more than 3 times that of a mycorrhizal one. The external P requirement of nonmycorrhizal cowpea is, however, 1/3 to 1/4 of that of nonmycorrhizal _L. leucocephala_, which is an extremely mycorrhizal-dependent species (11). Cowpea, therefore, can be considered as moderately dependent on VAM fungi. Mycorrhizal inoculation lowered the root/shoot ratio values of cowpea, an observation consistent with other's findings (1,2,22). The high root/shoot ratio of plants observed in the unamended eroded soil indicates that the growth of shoot in these plants was severely curtailed because of the failure of the nonmycorrhizal roots to take up adequate levels of immobile nutrients, especially P, from the soil.

Despite declining of the percent infection of roots at high soil solution P level(>0.046 mg/l), the level of infection observed was never below 79%. This indicates the high tolerent limit of the fungus to elevated P levels. In an earlier study (11), the extent of colonization of leucaena root by the same fungus was not affected at the highest level of P tested in this study. Regulation of infection by soil solution P levels, therefore, appear to be influenced by host differences. Differences among VAM species could also affect the influence of soil solution P on the extent of root colonization and/or VAM activity (22).

The role of VAM fungi is, particularly, important in legumes because of the high requirement of P needed for the process of nitrogen fixation, and the fact that legumes are poor scavengers of soil P due to their restricted root system (18). Increase in nodulation due to mycorrhizal inoculation and P supply observed in this study are consistent with previous findings (1,23,24). The external P required for maximum nodulation in mycorrhizal cowpea was higher (0.046 mg/l) than that required for maximum dry matter production (0.026 mg/l). This phenomemon explains the commonly observed responsiveness of legumes to VAM inoculation and is reflective of the high demand of nodulation and nitrogen fixation for phosphorus. These results further underline the critical role that VAM symbiosis could play in the establishment of legumes on P-fixing and/or P-deficient soils such as eroded tropical soils. The very similar patterns observed in shoot P concentration and dry matter production indicates that plant growth was intimately related to P supply.

It can be concluded from these results that there are threshold, optimum and inhibitory soil solution P levels for the symbiotic interaction between the fungus G. aggregatum and cowpea. The results, thus, underline the importance of optimizing soil solution P levels for deriving maximum benefits from VAM inoculation.

Experiment 2

Mycorrhizal activity observed in cowpea or leucaena when grown in the eroded and uneroded soil was dependent on nutrient status of the soil samples. There appears to be a threshold level of P for mycorrhizal activity below which the activity was not shown. This view is supported by the fact that mycorrhizal activity was consistently low in the eroded soil unamended with P. The same conclusion could also be drawn from the shoot and root dry matter and shoot P status data which were significantly lower in the eroded soil than in the uneroded soil in the absence of added P. Plant growth in the two soils was similar after the addition of P.

Peak mycorrhizal activity measured in terms of changes in the P status of subleaflets or leaf discs was observed earlier in the uneroded soil than in the eroded soil when the soil samples were not amended with P. This indicates that the low P content in the eroded soil, possibly, retarted mycorrhizal development. Upon the addition of P to soil samples (0.026 mg/l), peak mycorrhizal activity was observed in both the eroded and uneroded soils at about the same time. Decrease in the extent of colonization of roots in the uneroded soil compared to that in the eroded soil could be due to the adverse influence of biological factors present in the uneroded soil. Such effect is less likely to occur in the eroded soil because of less microbial population.

Optimal P level for the symbiosis between leucaena and the endophyte observed in this study was similar to that observed by Habte and Manjunath (11) in a fumigated soil. Since the maximum shoot dry weights of leucaena and cowpea were reached at the soil solution P levels of 0.026 and 0.046 mg/l, respectively, the external P requirement of mycorrhizal cowpea appears to be greater than that of leucaena. A similar external P requirement for mycorrhizal leucaena has been reported by Habte and Manjunath (11).

Erosion treatment does not seem to have a major effect on nodule dry matter production in leucaena, but in cowpea the nodule dry weight was lower in the eroded soil than in the uneroded soil when not fertilized with P. Nodulation in cowpea, is probably, more sensitive to erosion-induced P deficiency. This is also indicated from the fact that nodule dry matter production in cowpea increased almost linearly with the application of P upto 0.046 mg/l. In general, the similarity observed in the trends of root colonization, shoot P status, and dry matter yields emphasizes the importance of VAM fungi in the P nutrition and growth of plants in eroded soils. Thus, eroded soils, when inoculated with VAM fungi and fertilized with P, could have productivity similar to the uneroded soil.

The results of this experiment emphasize the necessity for amending eroded tropical soils with P in order to achieve maximum symbiosis between legumes and VAM fungi. The results also indicate the existence of a threshold and optimal levels of soil solution P for the symbiotic interaction between the fungus _G. aggregatum_ and leucaena or cowpea.

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CHAPTER 6

OPTIMIZATION OF LIME, ORGANIC RESIDUE AND MOLYBDENUM FOR REHABILITATING ERODED SOIL THROUGH MYCORRHIZAL INOCULATION

INTRODUCTION

Among the various soil factors that influence plant growth, pH is an important one. The growth of leguminous plants has been shown to behave differently at different pH (11). Since soil erosion causes a reduction in pH (See Appendix A), the growth of sensitive plants in such soils is likely to be curtailed. The use of VAM fungi in establishing leguminous species in eroded soils appears to be promising, but there are two main problems that need to be overcome. Firstly, mycorrhizal spore germination and also, to some extent, host-endophyte association is influenced by soil pH (1,4,15). Secondly, leguminous plants have preference for certain pH ranges (11). So, in order to derive maximum benefits from mycorrhizal inoculation, liming would appear to be one of the necessary steps that must be considered.

Another important factor that influences plant growth is soil organic matter. Organic matter is the source of many plant nutrients. The influence of organic matter on mycorrhizal symbiosis is not clear. Hepper and Warner (6) demonstrated an increase in infectivity and growth of mycorrhizal clover when they amended their soil with organic material which consisted of sterile peat and organic matter isolated from the soil itself. The results of their work and those of other investigators (17,18) support the view that VAM fungi may grow saprophytically in soil, and emphasize the importance of organic matter for the VAM symbiosis. Eroded soils being deficient in organic matter may not, thus, be a good medium for supporting vigorous plant-VAM fungus interaction. Hence, it is hypothesized that amendment of organic residue to eroded soils will improve the infectivity and effectivity of VAM fungi.

Molybdenum is also an important nutrient that is needed for plant growth, especially for N_2-fixation (2,10). The importance of molybdenum in N_2-fixation lies in the fact that it is a constituent of the enzyme "nitrogenase", which catalyzes the actual N_2 reduction process. Since soil erosion brings about a decrease in Mo content of soil (See Appendix A), it is anticipated that the growth and nodulation of leguminous species as well as the process of N_2-fixation would be impaired in that soil. In order to establish N_2-fixing and mycorrhizal leguminous plants on eroded soils, measures must be taken to ensure that the symbiotic processes involved are not limited by the inadequacy of Mo. Hence the amounts of Mo to be applied to eroded and uneroded soils must be accurately determined.

The objective of this study was to determine the optimum levels of lime, organic residue and/or molybdenum necessary for establishing N_2-fixing and mycorrhizal cowpea and leucaena in an eroded soil.

MATERIALS AND METHODS

Experiment 1: Determination of optimum level of lime necessary for establishing cowpea and leucaena in eroded soil

Based on a lime requirement curve [Fig. A.1 (Appendix A)], pH levels were established in the eroded and uneroded soils. Treatments consisted of plants grown in the eroded or uneroded soil at the original pH level (5.4 for the eroded and 5.9 for the uneroded soil) or at the pH levels of 6.0 (the pH level of the uneroded soil) and 6.5. Leaf disc or subleaflet samples were taken for P determination every 5 days begining at 12 days after planting (DAP). Cowpea and leucaena were grown for 32 and 37 days, respectively. At harvest, measurements of colonization of roots by VAM fungi, shoot and root dry matter yield, nodulation and P content of shoots were determined.

Experiment 2: Determination of the optimum level of organic matter necessary for establishing cowpea and leucaena in eroded soil

Treatments consisted of plants grown in the eroded or uneroded soil unamended or amended with organic residue to give a range of organic matter concentrations. The native organic matter contents of the eroded and uneroded soils were 2.15 and 3.69 percent, respectively. Thus the target organic matter levels in the eroded soil were 2.15, 3.69 and 7.38 percent while those of the uneroded soil were 3.69 and 7.38 percent. The organic residue added to the soils consisted of dried leucaena leaves and twigs that were ground to pass 1 mm aperture size sieve. Total N and P contents of the ground plant material were 4.05 and 0.20 percent, respectively. Required amounts of the organic residue were added to the soil samples and mixed well before planting. Leaf disc or subleaflet samples were taken for P determination every 5 days begining at 12 days after planting. Cowpea and leucaena were grown for 32 and 37 days. At harvest, measurements of colonization of roots by VAM fungi, shoot and root growth, nodulation and P content of shoots were determined.

Experiment 3: Determination of the optimum level of molybdenum necessary for establishing cowpea and leucaena in eroded soil

Treatments consisted of plants grown in eroded or uneroded soil unamended or amended with Mo at the rate of 2.2, 4.4 and 6.6 Kg/ha. Molybdenum was added to soil as a solution of $Na_2MoO_4.2H_2O$. Leaf disc or subleaflet samples were taken for P determination every 5 days begining at 12 days after planting. Cowpea and leucaena were grown for 32 and 37 days. At harvest, measurements of colonization of roots by VAM fungi, shoot and root growth, nodulation and P content of shoots were determined.

RESULTS AND DISCUSSION

Response of cowpea and leucaena to liming of eroded and uneroded soil

Cowpea. When the pH of the eroded soil was raised from 5.4 to 6.0 there was a significant increase in the extent of colonization of roots of cowpea by VAM fungi (Fig. 6.1). A further increase in pH led to a decrease in the level of colonization which was not significant.

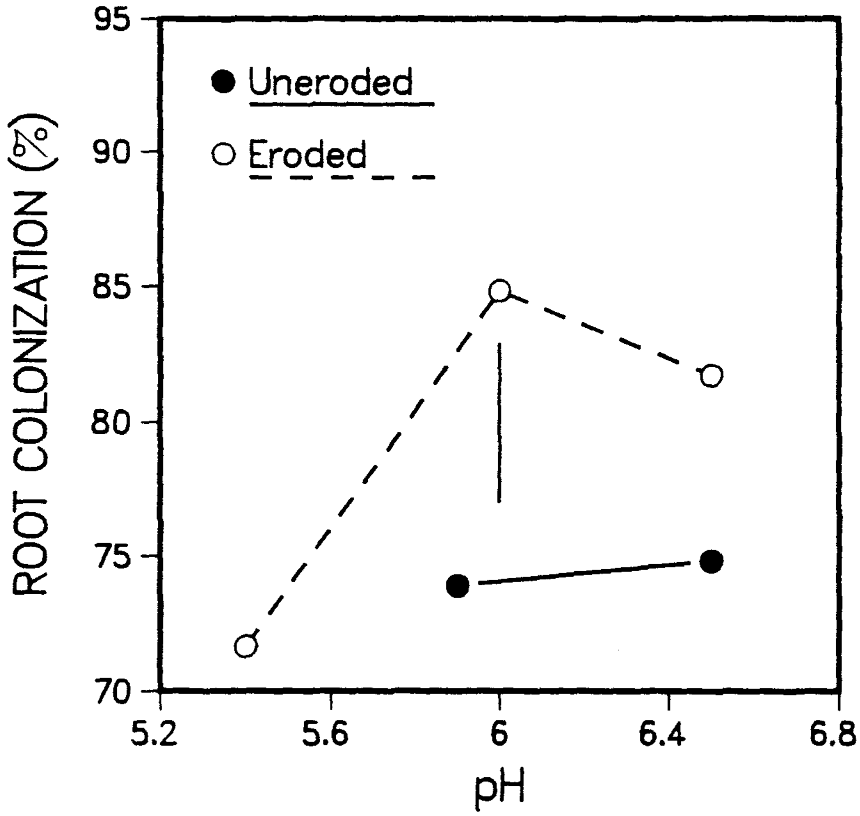

FIG. 6.1. The influence of liming on the extent of VAM colonization of cowpea roots in uneroded or eroded soil inoculated with G. aggregatum. Vertical bar represents LSD at the 5% level.

In the uneroded soil, pH did not significantly influence VAM colonization of roots. Some of the work done before have indicated that spore germination of endophytes, which also reflects their infectivity, is dependent to some extent on soil pH (1,4). These were, however, incubation studies conducted in petri plates using sterile soil, soil extract agar or water agar. These results, thus, can not be compared with the results of the present study. Apart from spore germination, the symbiotic effectiveness of different endophytes vary at different soil pH (5,15). In these studies, several species of VAM fungi were tested for symbiotic effectiveness at different pH using sterile soils whereas in the present study the effectivity of only one species was studied in nonsterile eroded and uneroded soils. So, the optimum pH observed in my study for the symbiotic interaction between leucaena and G. aggregatum can not be compared with the optimum pH observed in the above mentioned studies involving different soils, hosts and endophytes. The lower level of root colonization observed in the uneroded soil than in the eroded soil is probably due to higher populations of soil microorganisms in the uneroded soil that might contain biological factors harmful to the germination of spores or colonization of roots. Gianinazzi-Pearson and Diem (3) and St. John and Coleman (16) in their reviews mentioned about the harmful effects of organisms (e.g. actinomycetes) and nematodes on VAM infection level and spore production. Spores of certain VAM fungi can often be parasitized by some hyperparasitic fungi and there could also be certain soil animals such as soil mites of the super family Cryptostigmata that eat mycorrhizal hyphae (16). Thus the spread and colonization of VAM fungi are likely to be more favored in the eroded soil where there are less chances for interference from antagonistic biological factors.

The changes in mycorrhizal activity monitored in terms of leaf disc P content of cowpea grown at different pH levels in the eroded and uneroded soils are shown in Fig. 6.2. Leaf disc P content of plants grown in the eroded soil was about 6 g at 12 DAP and remained at about the same level until 17 DAP. Thereafter, the P content of leaf discs increased, reaching peak values at 22 DAP and then declined to about the original level. No significant difference in mycorrhizal activity was observed as a result of liming the eroded soil. Similar were the results in the uneroded soil. Trends observed when mycorrhizal activity was monitored in terms of the P concentration of leaf discs were also similar [Fig. B.4 (Appendix B)]. Based on the above results it can be concluded that liming has no effect on mycorrhizal activity in cowpea.

There was no change in shoot P concentration of cowpea after liming in either soil treatments but total shoot P content was significantly higher when the eroded soil was limed up to pH 6.0 compared to the unlimed soil (Table 6.1). This increase in shoot P content is most likely a consequence of increase in shoot dry weight as pH was raised from 5.4 to 6.0 in the eroded soil. No such increase in P uptake was observed in the uneroded soil.

There was also no significant increase in nodule dry matter production of cowpea due to liming, although nodule dry weight was greater in the limed soil than in the unlimed one (Table 6.1). Munns

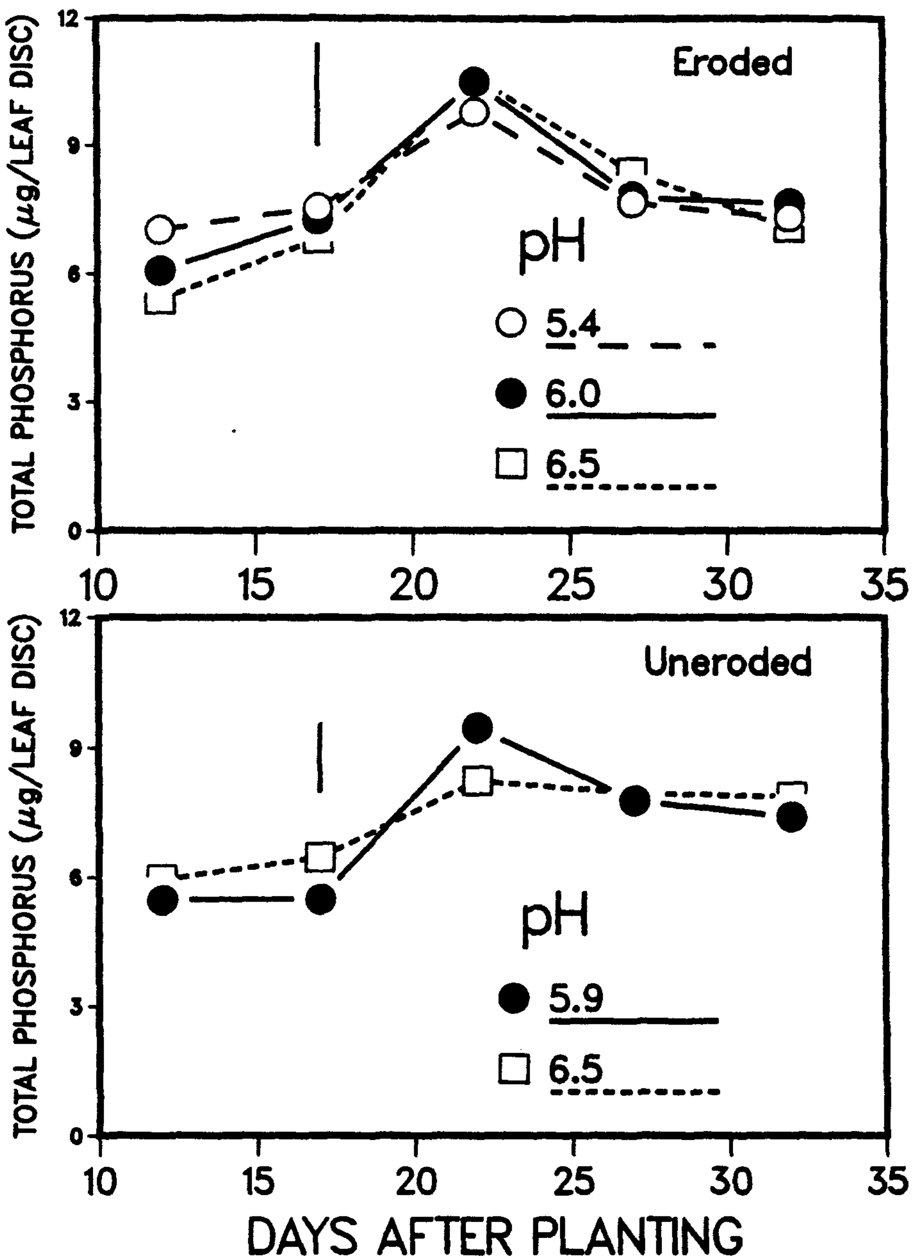

FIG. 6.2. The influence of liming on the development of mycorrhizal effectiveness in cowpea grown in eroded or uneroded soil inoculated with G. aggregatum. Vertical bars represent LSD at the 5% level.

TABLE 6.1. Influence of liming on nodule dry weight, root/shoot ratio and shoot P status of cowpea grown in eroded or uneroded soil inoculated with *G. aggregatum*[1]

Lime added	Nodule dry weight (g)	Root/shoot ratio	Shoot P conc. (%)	Total shoot P (mg)
		Eroded Soil		
No lime added (pH 5.4)	0.4125a	0.17a	0.2275a	46.08b
Lime added to pH 6.0	0.4539a	0.14b	0.2602a	60.19a
Lime added to pH 6.5	0.4331a	0.12b	0.2479a	55.37ab
		Uneroded Soil		
No lime added (pH 5.9)	0.3580a	0.13b	0.2261a	51.64ab
Lime added to pH 6.5	0.4106a	0.12b	0.2382a	57.79ab

[1]Means followed by the same letter within a column are not significantly different at the 5% level.

et al. (12) also did not observe any increase in nodulation of cowpea due to liming.

Shoot dry matter production of cowpea was significantly lower in the eroded soil than in the uneroded soil in the absence of lime (Fig. 6.3). When the eroded soil was limed to pH 6.0, shoot dry weight increased significantly and the difference in shoot dry weight that was observed between the two soil samples was eliminated. There was no further increase in shoot dry weight as the pH was raised. In the uneroded soil, on the other hand, pH had no signficant influence. Root dry matter production decreased with increase in pH in the eroded soil while there was no change in the uneroded soil (Fig. 6.3). The decrease in dry matter production of cowpea as pH was elevated above 6.0 contradicts the findings of Munns and Fox (11), who observed maximum yield at about pH 6.5. On the other hand, Lowther and Adams (9) have reported growth depression of white clover due to excessive liming of soil at the rate of 40 or 160 cwt/acre. The explanation suggested for this depression in growth was lime induced phosphorus deficiency. The discrepancy between the results of this study and that of Munns and Fox (11) could be due to changes in soil solution P status in the limed soil that could have arisen if the P sorption isotherm was not done with the limed soil. Munns and Fox did not specify whether the P sorption isotherm was based on the limed or unlimed soil. Furthermore, Munns and Fox used a different species of cowpea (Vigna sinensi). It is known that different leguminous species behave differently at different pH levels (11). The root/shoot ratio of cowpea was highest when it was grown in the unlimed eroded soil

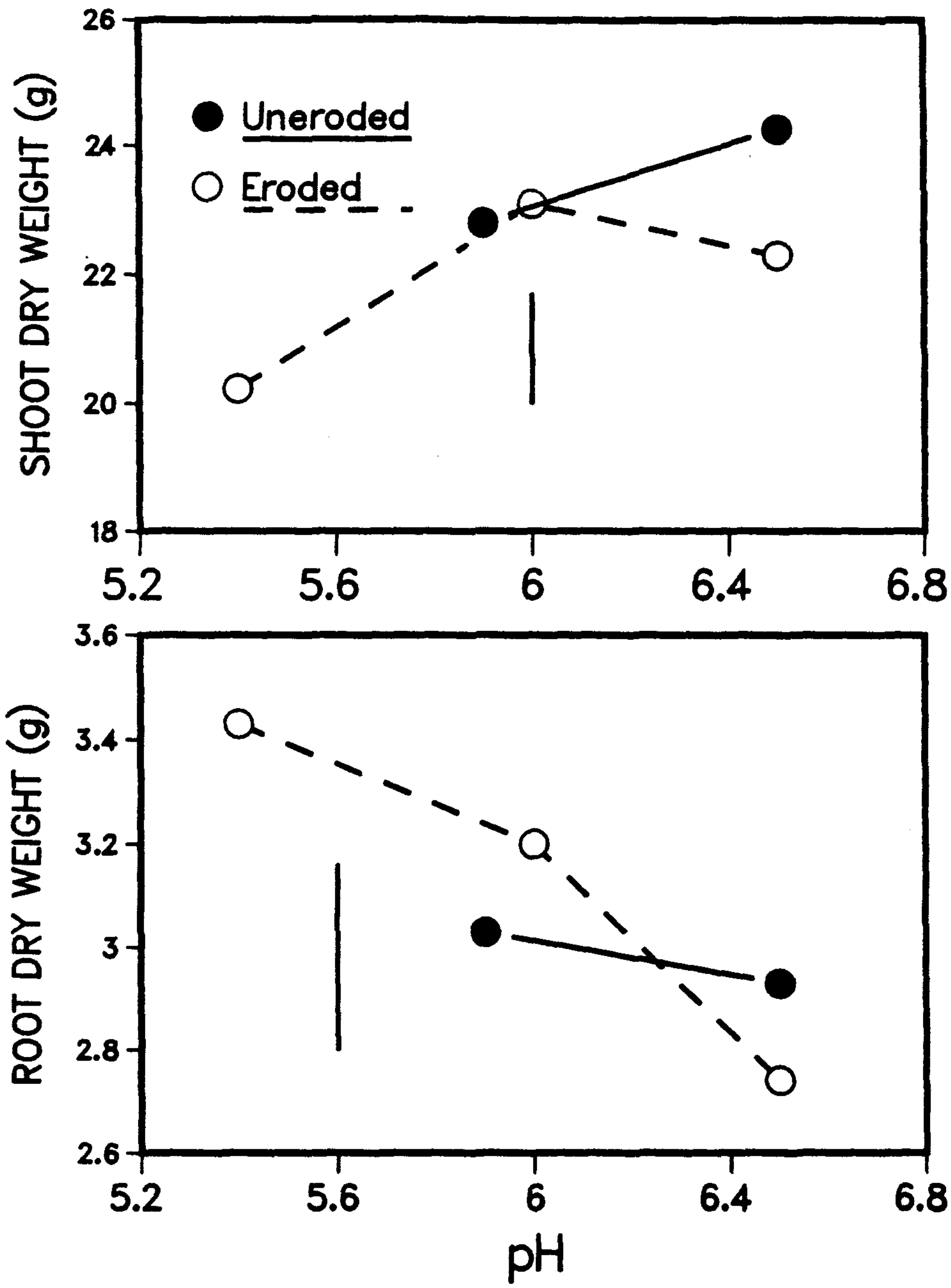

FIG. 6.3. The influence of liming on dry matter production of cowpea grown in uneroded or eroded soil inoculated with *G. aggregatum*. Vertical bars represent LSD at the 5% level.

(Table 6.1). The ratio decreased significantly when the soil was limed. There was, however, no change in the root/shoot ratio of cowpea grown in the uneroded soil. High root/shoot ratio observed in the unlimed eroded soil indicate imposition of some stress on cowpea at the acidic pH of 5.4.

The results of this study indicate that liming of the eroded soil was benecial to the growth of mycorrhizal cowpea despite no effect observed on mycorrhizal activity (leaf P status). Since shoot P concentration did not change as a result of liming, the increase in shoot dry matter yield observed is, probably, due to factors other than the effect of liming on mycorrhizal activity or P uptake.

Leucaena. The extent of colonization of roots of leucaena was significantly lower in the eroded soil than in the uneroded soil in the absence of lime (Fig. 6.4). When the eroded soil was limed to pH 6.0, the extent of root colonization was increased significantly and reached a level higher than that observed in the uneroded soil. At higher pH levels the infection level decreased. In the uneroded soil, on the other hand, the extent of colonization of roots decreased after liming. Lower extent of colonization of roots in the uneroded soil than in the eroded soil, as explained in the previous experiment with cowpea, is probably due to the increased microbial activity in that soil that might contain biological factors antagonistic to VAM fungi.

The changes in mycorrhizal activity monitored in terms of P content of subleaflet of leucaena at different pH levels are depicted in Fig. 6.5. When the eroded soil was limed to pH 6.0, there was an increase in mycorrhizal activity at 17 DAP compared to that in the

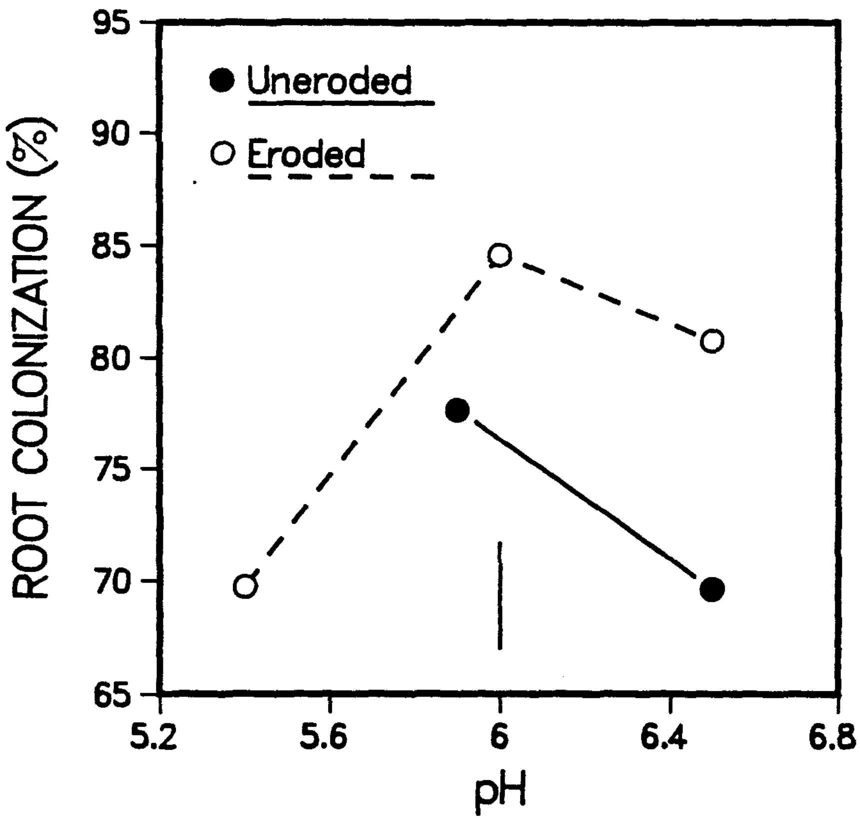

FIG. 6.4. The influence of liming on the extent of VAM colonization of roots of leucaena grown in uneroded or eroded soil inoculated with G. aggregatum. Vertical bar represents LSD at the 5% level.

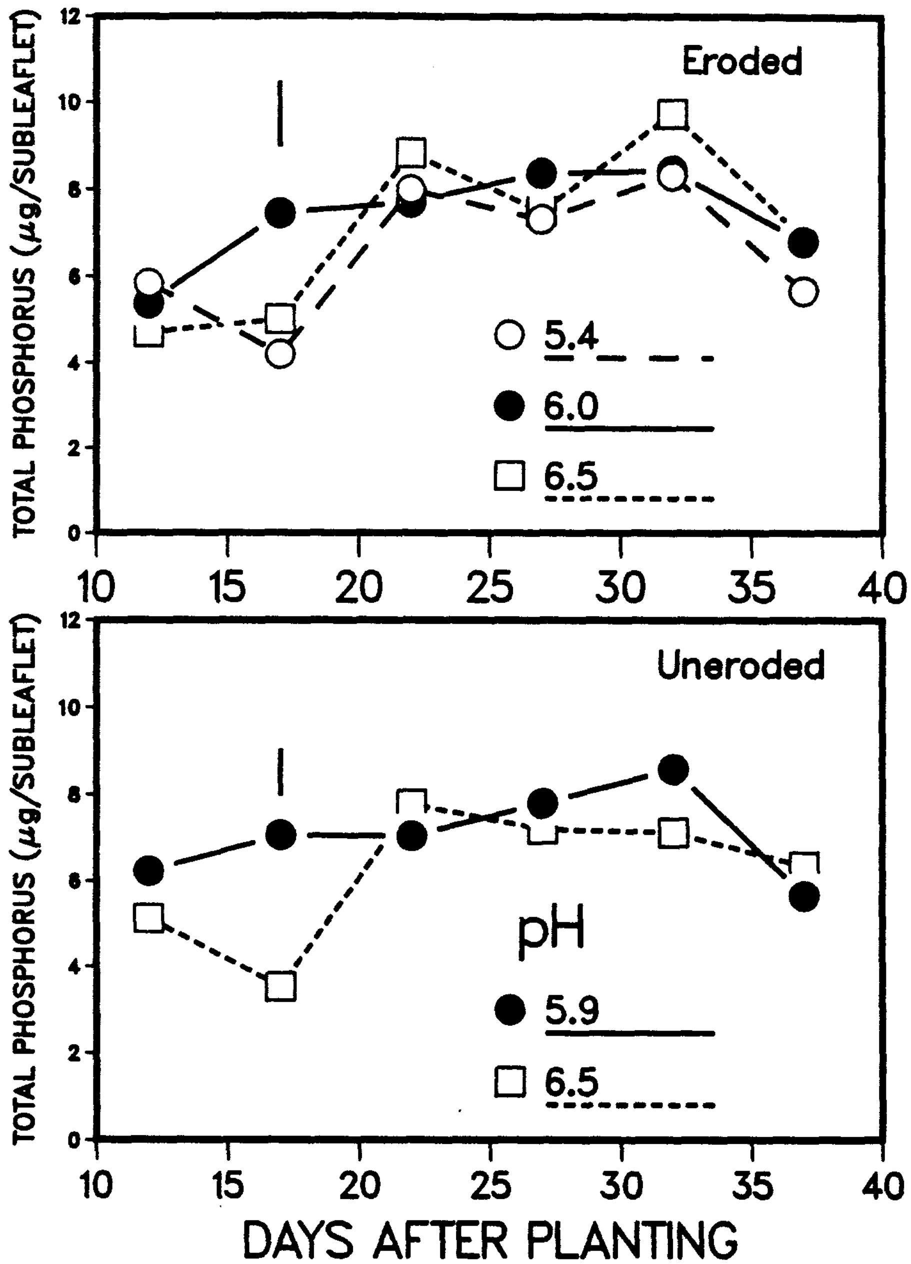

FIG. 6.5. The influence of liming on the development of mycorrhizal effectiveness in leucaena grown in eroded or uneroded soil inoculated with G. aggregatum. Vertical bars represent LSD at the 5% level.

unamended soil or the soil limed to pH 6.5. In the uneroded soil, liming upto pH 6.5 reduced the P content of leucaena subleaflet at 17 DAP compared to that in the unamended soil. This difference, however, disappeared at 22 DAP. The trends were similar when mycorrhizal activity was monitored in terms of the P concentration of subleaflets [Fig. C.3 (Appendix C)]. Based on these results, it seems that mycorrhizal activity, in general, was not significantly influenced by liming.

Shoot P content of leucaena was higher when grown in the limed eroded soil than in the unlimed one (Table 6.2). This increase could be due to increase in shoot dry weight as a result of liming the eroded soil. In the uneroded soil, lime had no effect on shoot P content. There was no significant increase in nodule dry weight due to liming (Table 6.2). Apparently the Rhizobium sp. used for inoculating the seeds was not responsive to increases in pH.

Shoot dry matter production of leucaena was significantly lower in the eroded soil than in the uneroded soil in the absence of lime (Fig. 6.6). When the eroded soil was limed to pH 6.0 (pH level in the uneroded soil), shoot dry weight increased significantly. Shoot dry weight values at this pH level were similar in the two soil samples. Liming the uneroded soil decreased shoot dry weight. Munns and Fox (11) studied the effect of lime on several tropical legumes and observed increases in shoot dry weight of leucaena even at pH 7.0, whereas in the present study growth was depressed at pH 6.5. As discussed earlier, this discrepency in result might be due to changes in soil solution P level as a result of not using the limed soil for

TABLE 6.2. Inflence of liming on nodule dry weight, root/shoot ratio and shoot P status of leucaena grown in eroded or uneroded soil inoculated with *G. aggregatum*[1]

Lime added	Nodule dry weight (g)	Root/shoot ratio	Shoot P conc. (%)	Total shoot P (mg)
		Eroded Soil		
No lime added (pH 5.4)	0.0735a	0.44ab	0.1911b	10.70b
Lime added to pH 6.0	0.0828a	0.49a	0.2053ab	13.40a
Lime added to pH 6.5	0.0823a	0.43ab	0.2148a	13.19a
		Uneroded Soil		
No lime added (pH 5.9)	0.0751a	0.36b	0.1968ab	14.19a
Lime added to pH 6.5	0.0842a	0.37b	0.2135ab	13.47a

[1]Means followed by the same letter within a column are not significantly different at the 5% level

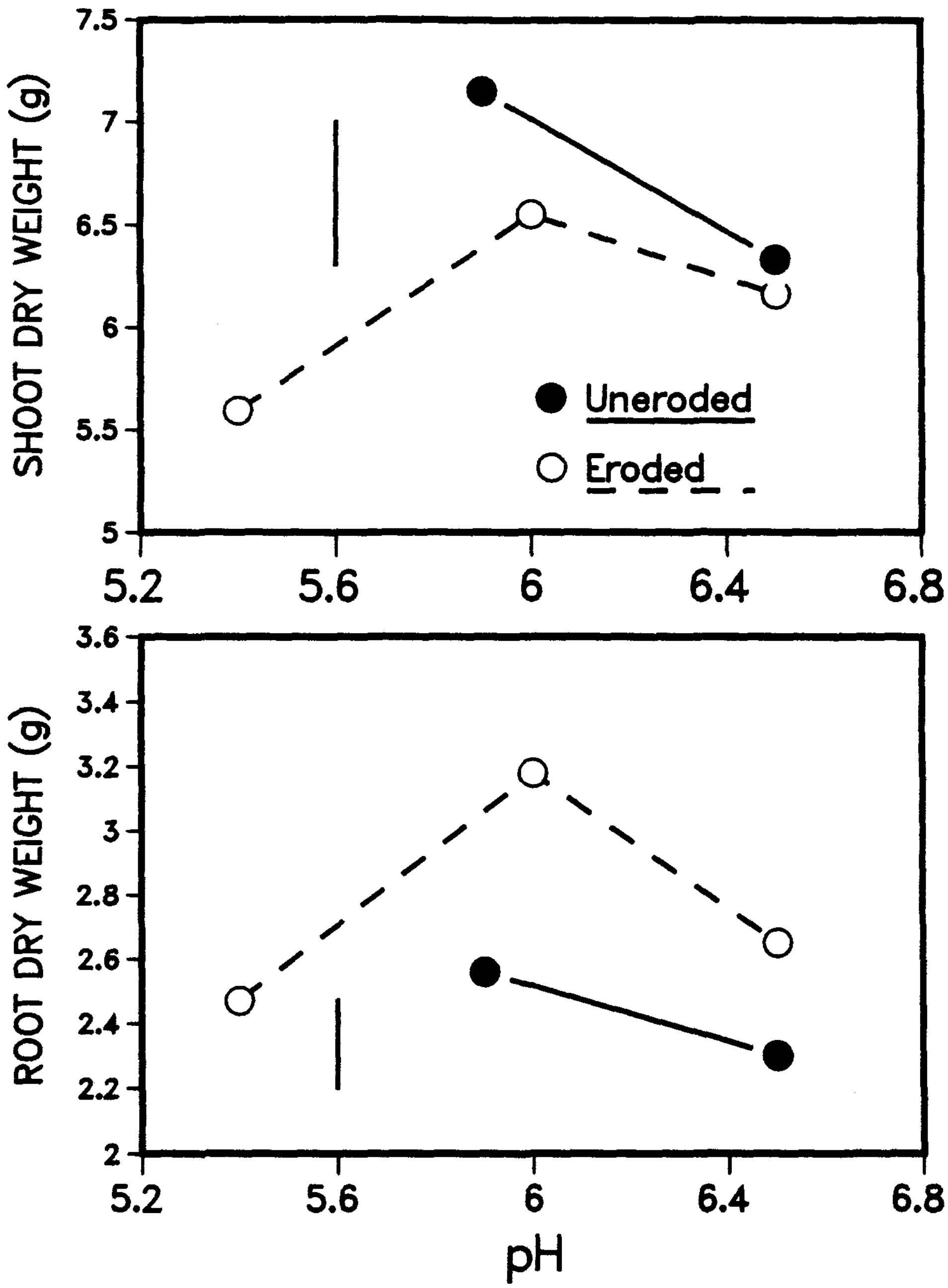

FIG. 6.6. The influence of liming on dry matter production of leucaena grown in uneroded or eroded soil inoculated with G. aggregatum. Vertical bars represent LSD at the 5% level.

establishing P sorption isotherm. Furthermore, the work of Munns and Fox was done in the field whereas the present experiment was conducted in greenhouse. Plants grown in fields are subject to many other factors. The trends observed for root dry matter production were similar to that observed for shoot dry matter production (Fig. 6.6). There was no significant difference in the root/shoot ratio of leucaena due to lime treatments (Table 6.2).

The results of this study indicate that liming of the eroded soil upto pH 6.0 was beneficial to mycorrhizal leucaena.

Response of cowpea and leucaena to amendment of organic residue in eroded and uneroded soil

Cowpea. Addition of organic residue to the soil samples did not significantly influence the extent of colonization of roots of cowpea (Table 6.3). These findings are contradictory to that of Hepper and Warner (6) who observed an increase in infectivity of soil by *G. mosseae* after the soil was amended with organic matter. Differences in the nature and extent of decomposition of the organic materials used and in the kind of host-endophyte associations involved might have contributed to the lack of agreement in the two studies.

Mycorrhizal activity was monitored in terms of the leaf disc P content of cowpea as a function of time in eroded and uneroded soils amended with different levels of organic residue. The results are given in Fig. 6.7. Mycorrhizal activity in the soil samples increased initially reaching peak values at 22 DAP and then levelled off. The activity did not appear to be significantly influenced by organic residue amendment. When mycorrhizal activity was monitored in terms

TABLE 6.3. Influence of organic residue on mycorrhizal infection level, nodule dry weight and shoot P status of cowpea grown in eroded or uneroded soil inoculated with *G. aggregatum*[1]

Organic residue added	Mycorrhizal infection level (%)	Nodule dry wt. (g)	Shoot P conc. (%)	Total shoot P (mg)
		Eroded Soil		
Not added (OM[a] 2.15%)	86.4a	0.3525a	0.2916a	69.41a
OM 3.69%	81.7a	0.3863a	0.3188a	79.30a
OM 7.38%	86.3a	0.2980a	0.3353a	78.68a
		Uneroded Soil		
Not added (OM 3.69%)	74.9a	0.3209a	0.2935a	64.78a
OM 7.38%	82.9a	0.2216a	0.3192a	78.08a

[1]Means followed by the same letter within a column are not significant at the 5% level

[a]OM = organic matter

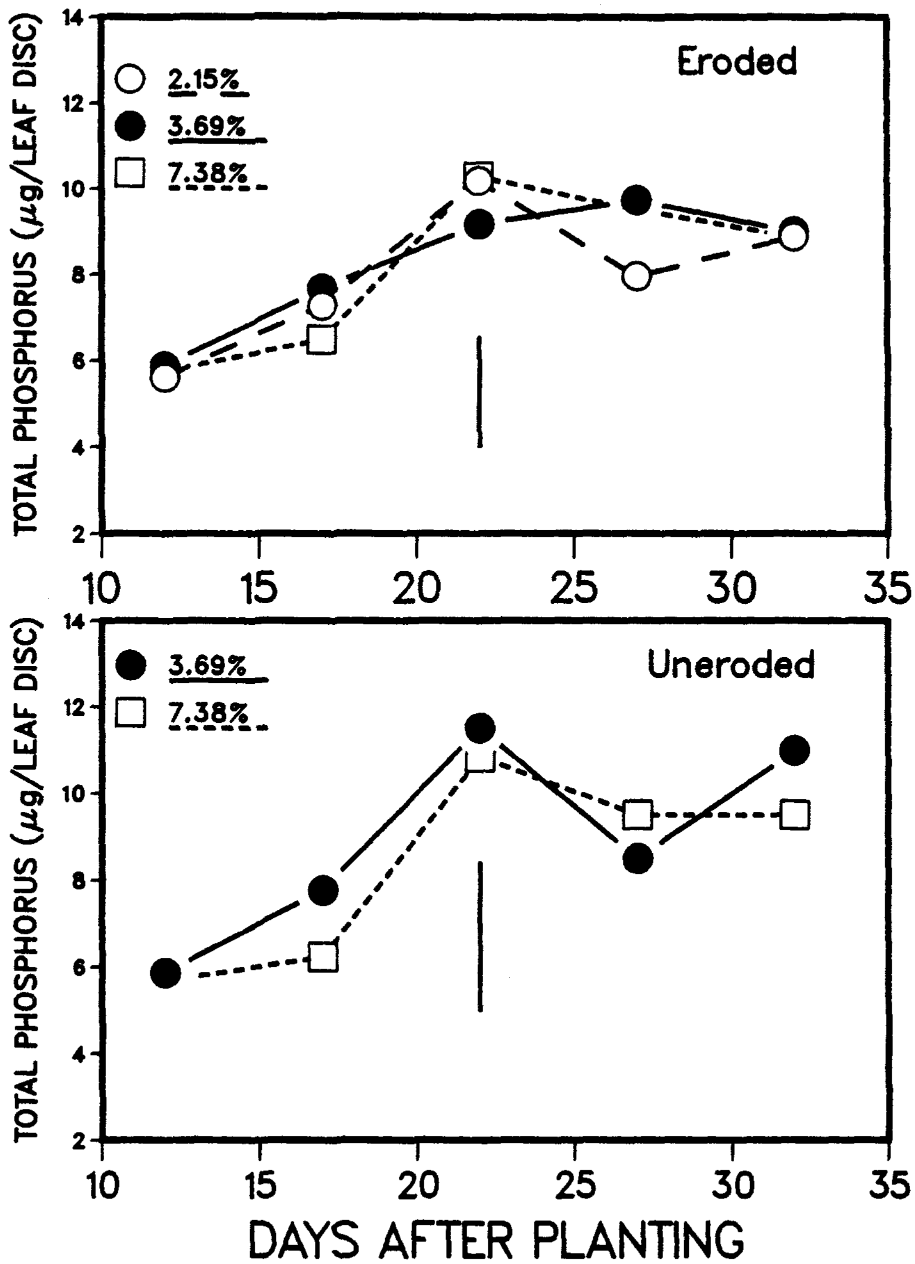

FIG. 6.7. The influence of organic residue on the development of mycorrhizal effectiveness in cowpea grown in eroded or uneroded soil inoculated with G. aggregatum. Vertical bars represent LSD at the 5% level.

of the P concentration of leaf discs, similar results were obtained [Fig. B.5 (Appendix B)].

Shoot P status (shoot P concentration and total shoot P) of cowpea was not affected by organic residue amendment (Table 6.3). Figure 6.8 illustrates the effect of organic residue on shoot Mn status of cowpea. Analysis of shoot Mn was done in order to determine the effect of organic residue on Mn availability to cowpea. The results show that there was a significant increase in Mn concentration of cowpea at the highest level of organic residue added. Hue (7) has shown some evidence which indicate that the decomposition of organic residues in soil release organic substances which tend to solubilize Mn.

Cowpea did not appear to respond significantly to the increase in tissue Mn level after organic residue amendment. Kang & Fox (8) demonstrated that the growth of young cowpea plants ceased at a Mn concentration of about 2600 ppm. They also demonstrated that Mn concentration of about 1000 ppm was not toxic to cowpea. The Mn level in plant tissue observed in this study was, thus, below the level that was toxic to cowpea. Nodule dry matter production and dry matter yield of cowpea were not affected by organic residue amendment (Fig. 6.9, Table 6.3).

The results of this study indicate that amendment of organic residue was not beneficial to mycorrhizal cowpea grown in the eroded soil.

Leucaena. Mycorrhizal colonization of roots was suppressed at the highest level of organic residue added (Table 6.4). Such a

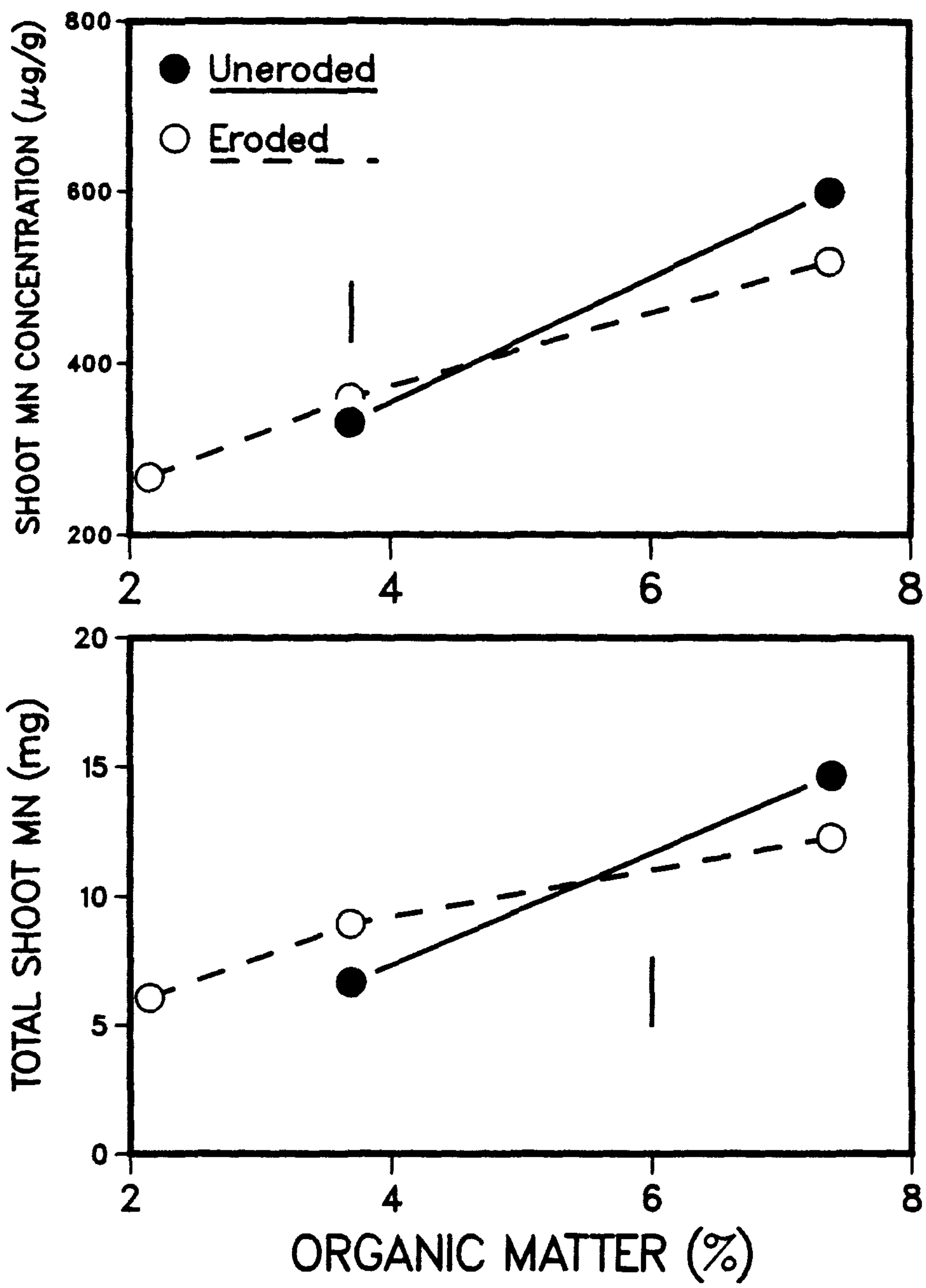

FIG. 6.8. The influence of organic residue on shoot Mn status of cowpea grown in uneroded or eroded soil inoculated with *G. aggregatum*. Vertical bars represent LSD at the 5% level.

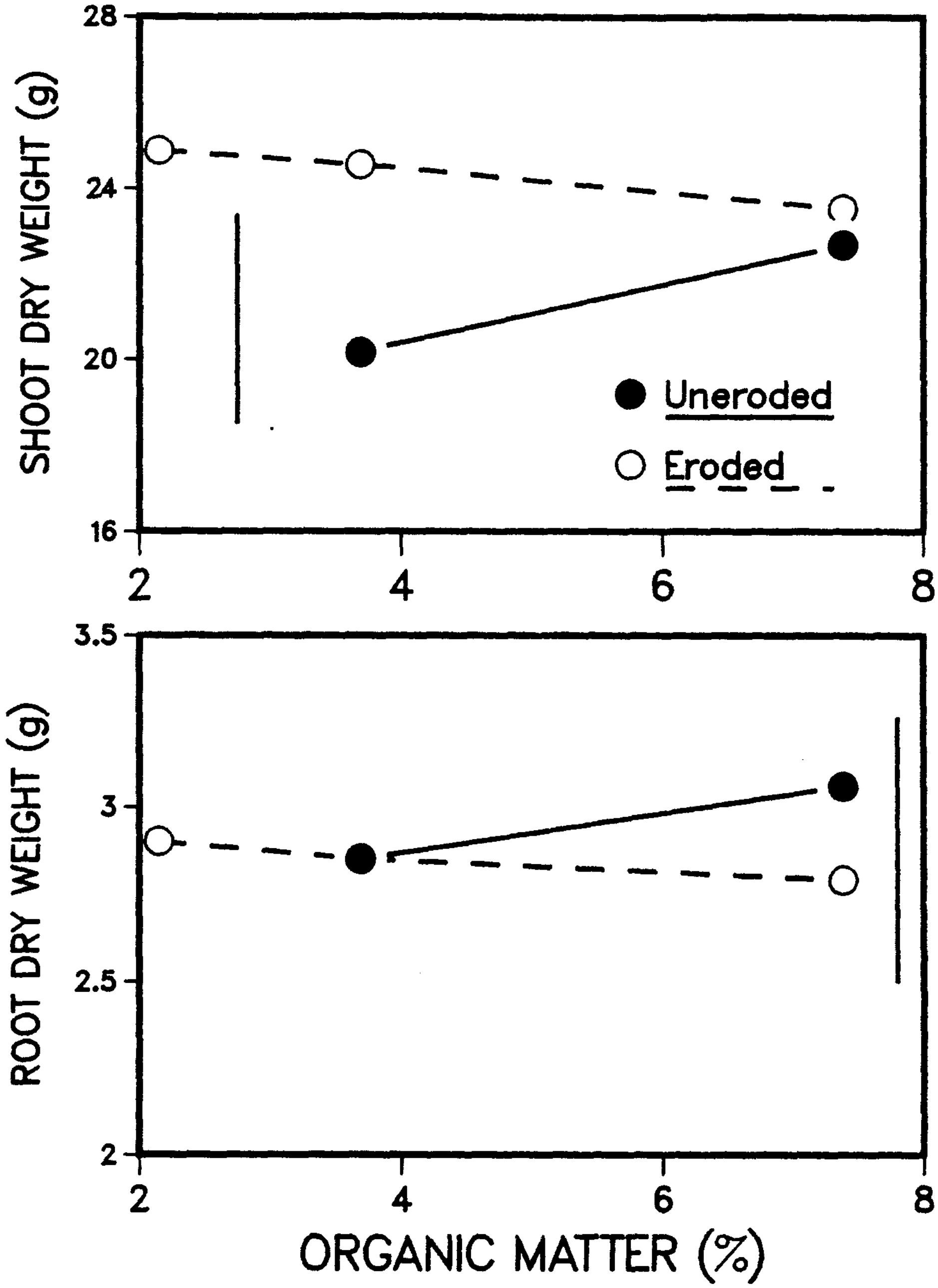

FIG. 6.9. The influence of organic residue on dry matter production of cowpea grown in uneroded or eroded soil inoculated with G. aggregatum. Vertical bars represent LSD at the 5% level.

TABLE 6.4. Influence of organic residue on mycorrhizal infection level, nodule dry weight and shoot P status of leucaena grown in eroded or uneroded soil inoculated with *G. aggregatum*[1]

Organic residue added	Mycorrhizal infection level (%)	Nodule dry wt. (g)	Shoot P conc. (%)	Total shoot P (mg)
		Eroded soil		
Not added (OM[a] 2.15%)	84.2a	0.0725a	0.2036a	12.31a
OM 3.69%	79.9ab	0.0713a	0.1949a	11.69a
OM 7.38%	73.3bc	0.0825a	0.1953a	9.09a
		Uneroded soil		
Not added (OM 3.69%)	81.6ab	0.0806a	0.2012a	13.04a
OM 7.38%	68.9c	0.0798a	0.2522a	12.87a

[1]Means followed by the same letter within a column are not significantly different at the 5% level

[a]OM = organic matter

suppression in mycorrhizal infectivity was not observed in the case of cowpea. Host species is, thus, appears to be a factor determining the influence of organic residue on mycorrhizal infectivity. The results of this study are also not in agreement with the findings of Hepper and Warner (6) who noticed an increase in mycorrhizal infectivity due to organic matter addition.

When mycorrhizal activity was monitored as a function of time in the eroded and uneroded soils amended with different levels of organic residue, the results presented in Fig. 6.10 were obtained. Mycorrhizal activity, in general, was low when the soil samples were amended with the highest level of organic residue tested (7.38%). Mycorrhizal activity was always higher in the uneroded unamended soil (native soil OM = 3.69%) compared to soil amended with 7.38% organic residue. In the eroded soil, mycorrhizal activity in most of the sampling periods was highest when amended with only 3.69% organic residue. Similar trends were observed when mycorrhizal activity was monitored in terms of the P concentration of subleaflets [Fig. C.4 (Appendix C)].

The decrease in root colonization at the high organic residue level was associated with a significant increase in shoot Mn status (Fig. 6.11). At the same time, the dry matter production of leucaena decreased significantly as the level of organic residue in the soil samples was raised to 7.38% (Fig. 6.12). There was, however, no change in dry matter production at 3.69% organic matter level.

Release of Mn in soil solution due to incorporation of organic residue in soil has been demonstrated by Hue (7). It was suspected

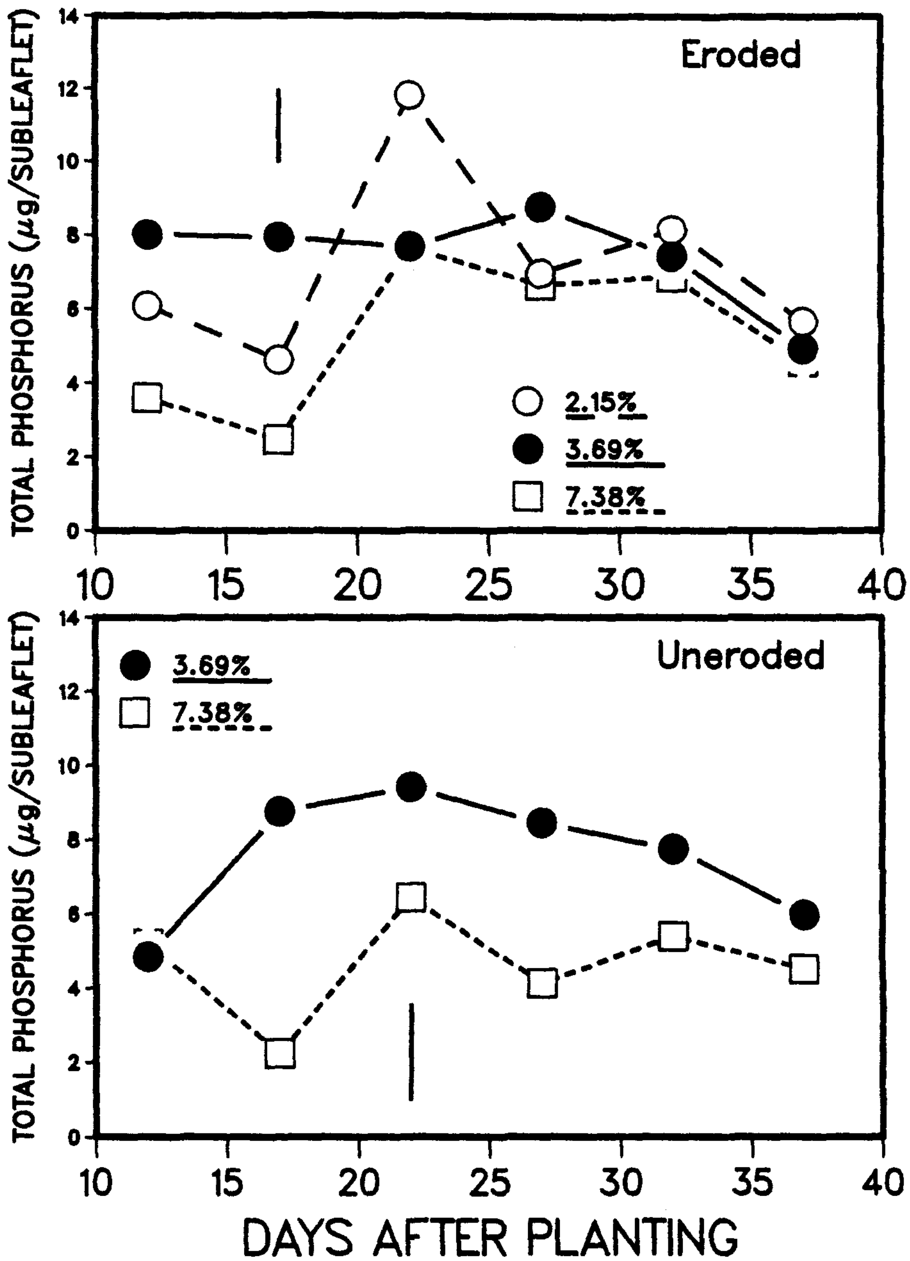

FIG. 6.10. The influence of organic residue on the development of mycorrhizal effectiveness in leucaena grown in eroded or uneroded soil inoculated with G. aggregatum. Vertical bars represent LSD at the 5% level.

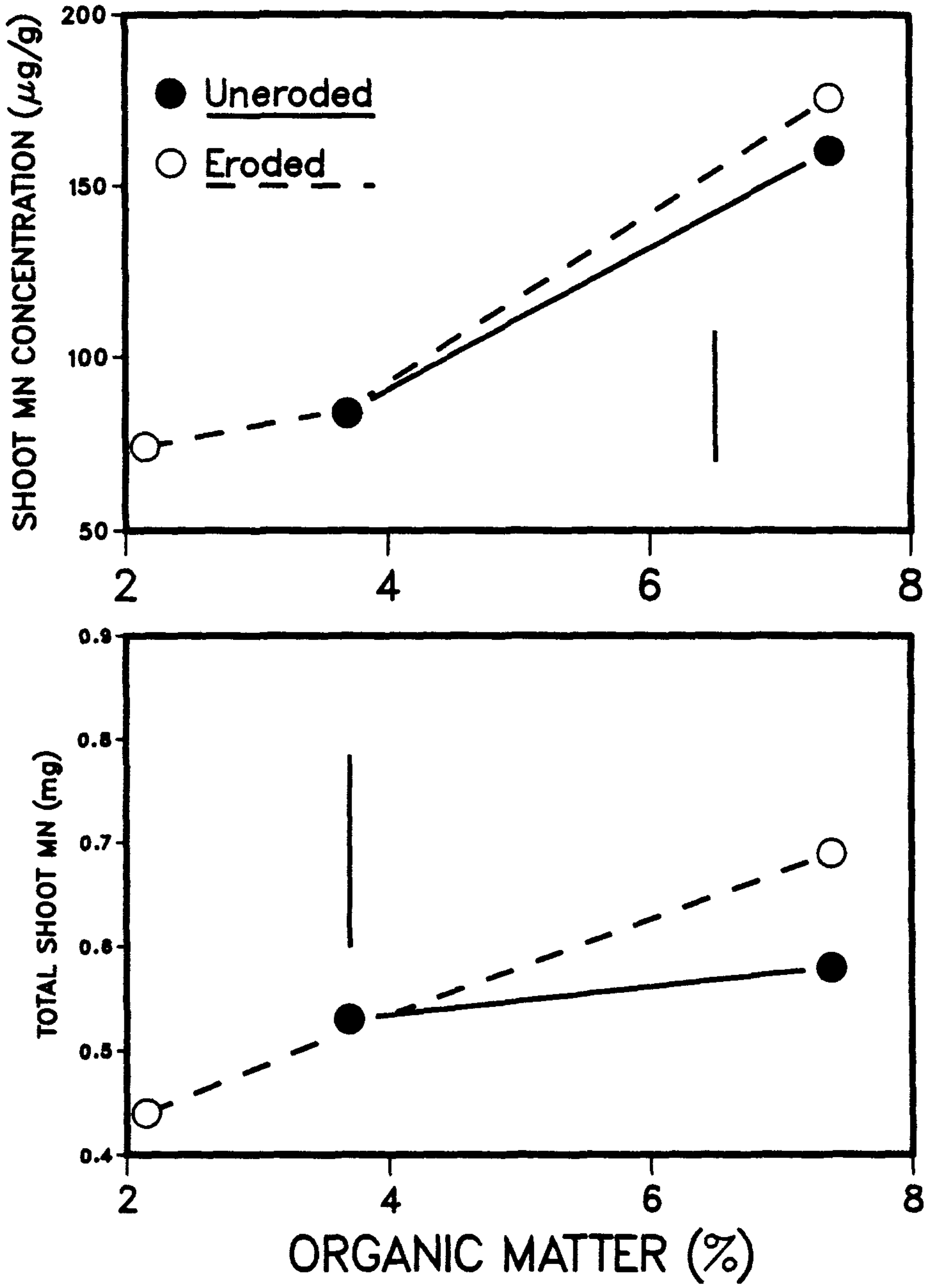

FIG. 6.11. The influence of organic residue on shoot Mn status of leucaena grown in uneroded or eroded soil inoculated with *G. aggregatum*. Vertical bars represent LSD at the 5% level.

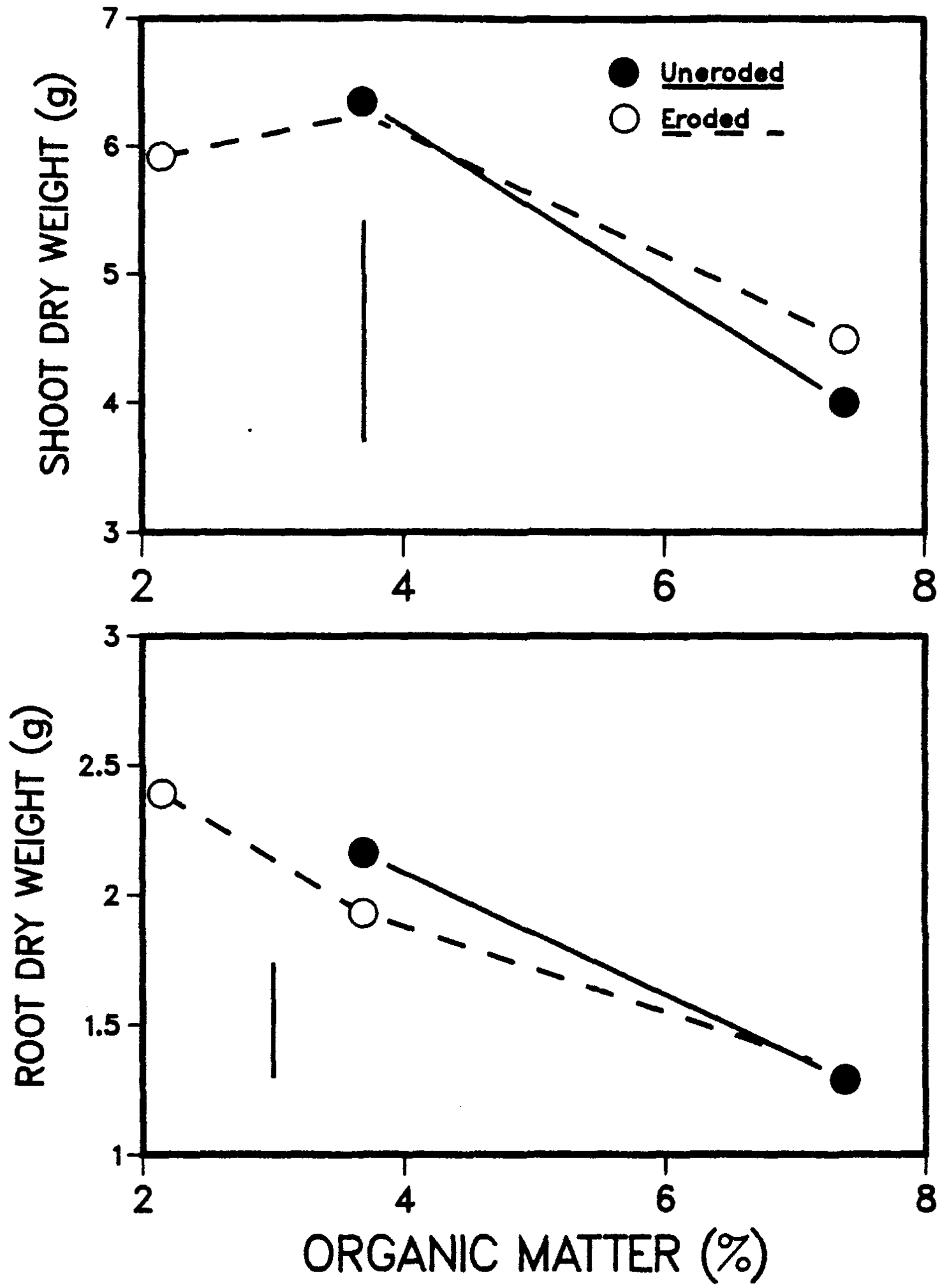

FIG. 6.12. The influence of organic residue on dry matter production of leucaena grown in uneroded or eroded soil inoculated with G. aggregatum. Vertical bars represent LSD at the 5% level.

that the growth depression in leucaena was due to Mn toxicity but the literature indicate that the toxic level of Mn for leucaena is 550 PPM (14) which is well above the highest level of Mn detected in leucaena tissue. So the growth depression in leucaena can not be explained in terms of Mn toxicity. Nitrogen deficiency due to immobilization of soil nitrogen as a result of amendment of organic residue can also be ruled out because of high N content of the organic residue used (4.05% N). One possible explanation could be induced iron deficiency because of organic materials. Organic materials are known to bind with iron thus reducing their availability.

There was no significant change in shoot P status and nodule dry matter production of leucaena as a result of organic residue amendment to the eroded and uneroded soil (Table 6.4).

The results of this experiment indicate that organic residue does not improve the growth of mycorrhizal leucaena in the eroded Wahiawa soil.

Response of cowpea and leucaena to amendment of molybdenum in eroded and uneroded soil

Cowpea. The extent of colonization of cowpea root by VAM fungi was increased due to Mo but the increase was not statistically significant (Table 6.5). Mycorrhizal activity monitored on the basis of P content of leaf discs was not influenced appreciably by amending the soil samples with different levels of Mo (Fig. 6.13). The activity, in general, increased slowly reaching a peak value at around 27 DAP and then declined. The increase in mycorrhizal activity observed at 17 DAP in the uneroded soil not amended with Mo is, most

TABLE 6.5. Influence of Mo on dry matter production, mycorrhizal infection level, nodulation and shoot P status of cowpea grown in eroded or uneroded soil inoculated with G. aggregatum[1]

	Molybdenum applied (Kg/ha)			
Measurements made	0	2.2	4.4	6.6
		Eroded Soil		
Mycorrhizal infection level (%)	71.7a	77.5a	75.6a	78.5a
Shoot dry weight (g)	15.30a	15.85a	15.52a	15.87a
Root dry weight (g)	2.40a	2.37a	2.27a	2.49a
Nodule dry weight (g)	0.6144a	0.6863a	0.6882a	0.6281a
Shoot P conc. (%)	0.2016a	0.2065a	0.2234a	0.2161a
Total shoot P (mg)	32.98a	32.89a	35.22a	34.38a
		Uneroded soil		
Mycorrhizal infection level (%)	70.1a	81.6a	74.4a	75.3a
Shoot dry weight (g)	15.69a	15.45a	15.52a	15.87a
Root dry weight (g)	2.24a	2.59a	2.12a	2.32a
Nodule dry weight (g)	0.6629a	0.6949a	0.7389a	0.6196a
Shoot P conc. (%)	0.1929a	0.2013a	0.2235a	0.2206a
Total shoot P (mg)	30.27a	31.03a	33.12a	31.32a

[1]Means followed by the same letter within rows for the same measurement in eroded and uneroded soil are not significantly different at the 5% level

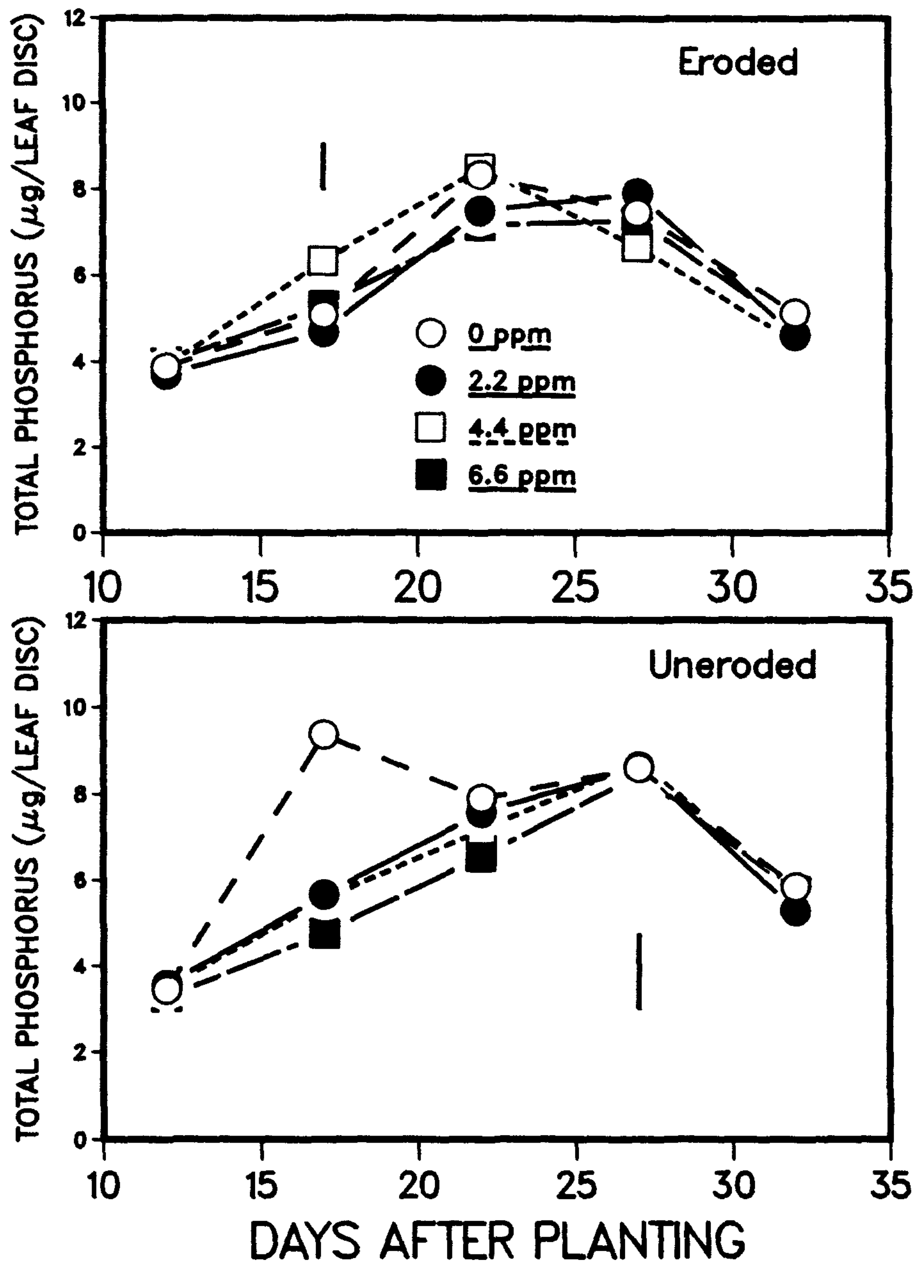

FIG. 6.13. The influence of Mo on the development of mycorrhizal effectiveness in cowpea grown in eroded or uneroded soil inoculated with G. aggregatum. Vertical bars represent LSD at the 5% level.

likely, a result of sampling error. The trends were similar when mycorrhizal activity was monitored in terms of the P concentration of leaf discs [Fig. B.6 (Appendix B)].

Nodule dry weight increased with increasing levels of applied Mo upto 4.4 Kg/ha, but the difference was not statistically significant (Table 6.5). The trends observed in concentration and uptake of shoot P were similar (Table 6.5). There was also no significant increase in dry matter production of cowpea due to Mo amendment of the eroded and uneroded Wahiawa soil (Table 6.5).

It is apparent from these results that the mycorrhizal cowpea was not significantly affected by Mo application to soil. Pongsakul (13) studied the influence of Mo application on the growth of 3 leguminous species in the Wahiawa soil. He observed that the growth of mycorrhizal stylosanthes was significantly improved when Mo was applied to the Wahiawa soil at the rate of 2 Kg/ha, whereas the growth of mycorrhizal desmodium and centrosema was not affected by Mo treatment. In my experiment, cowpea was not responsive to Mo application at the same pH level as that used by Pongsakul. The Mo requirement of cowpea is probably quite low and the amount of Mo available in the limed Wahiawa soil is, perhaps, adequate enough for the normal growth of cowpea. Pongsakul (13) also did not observe any increase in root colonization and nodule dry weight of mycorrhizal plants when he applied Mo to uneroded Wahiawa soil at pH 6.0.

Based on the results of this study, it appears that Mo does not play an important role in improving the growth of mycorrhizal cowpea in the eroded Wahiawa soil.

Leucaena. The extent of colonization of leucaena roots increased with increasing levels of Mo (Table 6.6). This observation was contradictory to that of Pongsakul (13) who did not observe any increase in root colonization due to Mo application. The contradiction of my observatiion to that of Pongsakul could be a result of different species used by him. The extent of root colonization observed in this study was higher in the eroded than in the uneroded soil. This is probably because of the lower level of organisms in the eroded soil that may suppress root colonization or spore germination.

Mycorrhizal activity monitored in terms of P content of leucaena subleaflets as a function of time was quite similar to that observed for cowpea (Fig. 6.14). In general, Mo did not significantly influence mycorrhizal activity in the eroded and uneroded soil samples. The results were similar when mycorrhizal activity was monitored in terms of the P concentration of subleaflets [Fig. C.5 (Appendix C)].

Dry matter production and nodule dry weight values were not significantly influenced by treatment of soil with Mo (Table 6.6). However, shoot P uptake of leucaena was significantly increased when the soil samples were amended with Mo (Table 6.6). This increase in shoot P uptake is, probably, due to the combined effect of increase in shoot dry matter production and shoot P concentration as a result of Mo application, which themselves were not significantly different.

TABLE 6.6. Influence of Mo on dry matter production, mycorrhizal infection level, nodulation and shoot P status of leucaena grown in eroded or uneroded soil inoculated with *G. aggregatum*[1]

Measurements made	Molybdenum applied (Kg/ha) 0	2.2	4.4	6.6
	Eroded soil			
Mycorrhizal infection level (%)	68.0bc	76.3abc	79.9a	79.0ab
Shoot dry weight (g)	1.89b	2.40ab	2.20ab	2.30ab
Root dry weight (g)	0.72a	0.76a	0.77a	0.80a
Nodule dry weight (g)	0.1691a	0.1957a	0.1886a	0.1802a
Shoot P conc. (%)	0.2359a	0.2452a	0.2491a	0.2489a
Total shoot P (mg)	4.45e	5.82bcd	5.42cd	5.65cd
	Uneroded soil			
Mycorrhizal infection level (%)	69.1abc	67.2c	71.6abc	68.5abc
Shoot dry weight (g)	2.13ab	2.54ab	2.78a	2.60a
Root dry weight (g)	0.68a	0.76a	0.73a	0.78a
Nodule dry weight (g)	0.1762a	0.1905a	0.1880a	0.1789a
Shoot P conc. (%)	0.2504a	0.2704a	0.2663a	0.2558a
Total shoot P (mg)	5.32de	6.83ab	7.40a	6.58abc

[1]Means followed by the same letter within rows for the same measurement in eroded and uneroded soil are not significantly different at the 5% level

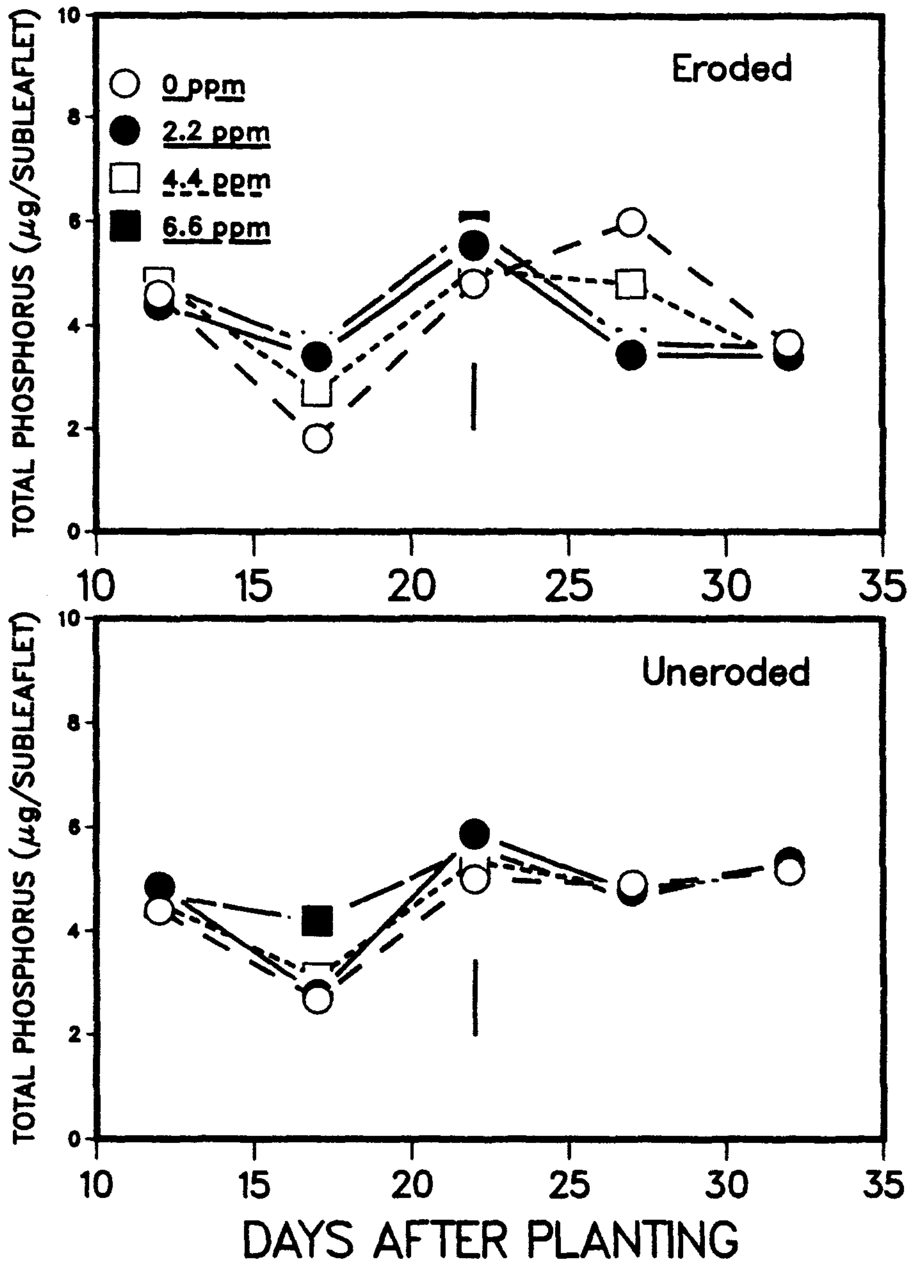

FIG. 6.14. The influence of Mo on the development of mycorrhizal effectiveness in leucaena grown in eroded or uneroded soil inoculated with G. aggregatum. Vertical bars represent LSD at the 5% level.

The results of this experiment were similar to the results obtained in the experiment done involving cowpea. It, therefore, appears that application of Mo to the eroded soil does not, in general, influence the growth of mycorrhizal leucaena.

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CHAPTER 7

OPTIMIZATION OF INORGANIC NITROGEN FOR REHABILITATING ERODED SOIL THROUGH MYCORRHIZAL INOCULATION

INTRODUCTION

Leguminous plants are unique among other plants because of their ability to fix atmospheric nitrogen through symbiotic association with *Rhizobium*. This ability to fix atmospheric nitrogen is affected by many soil factors. One of the important factors that affects N_2-fixation in legumes is the inorganic N content of soil (9,10,19). Apart from the symbiotic association with rhizobia, legumes can also form an association with vesicular-arbuscular mycorrhizal (VAM) fungi. This tripartite association is important because the extra P that is made available by VAM fungi can be used to meet the high demand of P required for N_2-fixation.

Erosional loss of soil is often associated with loss of nutrients including N. If the loss of N is severe, it may affect the symbiosis between legume, VAM and *Rhizobium*. The activity of VAM fungi has been shown to be influenced by the N content of soils (3,16). Eroded soils being low in mycorrhizal activity apart from N content may be rehabilitated with legumes if inoculated with efficient strains of VAM fungi and amended with proper levels of nitrogen.

The objective of this study was to determine the level of inorganic N necessary for the growth of mycorrhizal and nodulated cowpea and leucaena in an eroded Oxisol.

MATERIALS AND METHODS

Cowpea or leucaena was grown in the eroded or uneroded soil amended or unamended with inorganic N. The rates of inorganic N were 0, 25, 50 and 100 ppm. The source of nitrogen was NH_4NO_3 which was added as a solution. Phosphorus was added to obtain a concentration of 0.026 mg/l in solution. A blanket application of other nutrients was also added as described in Chapter 2. Cowpea and leucaena were grown for 32 and 37 days, respectively. Every 5 days, begining on 12 days after planting (DAP) the phosphorus content of leaf discs of cowpea or subleaflets of leucaena was determined. After harvest, the extent of colonization of roots by VAM fungi, shoot, root and nodule dry matter, shoot phosphorus and nitrogen contents were determined. Analysis of tissue N was done by digesting the samples using the salicylic acid modification of the Kjeldahl procedure (4). The digests were then submitted to the Diagnostic Service Center, Department of Agronomy and Soil Science, University of Hawaii for colorimetric determination of N by the Indophenol blue method (1).

RESULTS

Cowpea. The extent of colonization of roots by *G. aggregatum* in the absence of added N was significantly higher in the eroded soil than in the uneroded soil (Fig. 7.1). This difference ceased to be significant when N was added at the rate of 50 ppm. In the uneroded soil, there was a significant increase in the extent of root colonization when the soil was amended with 25 ppm N. The activity was maintained upto 50 ppm, but increased rapidly when 100 ppm of inorganic N was added. In the eroded soil, infection level was significantly influenced only at the highest N level.

Mycorrhizal activity monitored in terms of the P content of leaf discs of cowpea grown in the eroded and uneroded soils amended with different levels of inorganic N is illustrated in Fig. 7.2. In the absence of added N, mycorrhizal activity was higher in the eroded soil than in the uneroded soil initially. In general, the activity increased with time, reaching peak values at about 22-27 days after planting. The initial depression in activity that was observed in the uneroded soil not amended with N disappeared when N was added at the rate of 25 ppm. Higher levels of N did not seem to have clear cut influence on mycorrhizal activity. Similar trends were observed when mycorrhizal activity was monitored in terms of the P concentration of leaf discs [Fig. B.7 (Appendix B)].

Shoot P concentration of cowpea grown in the eroded and uneroded soils did not differ significantly from each other regardless of N amendments (Fig. 7.3). There was almost a linear increase in shoot P

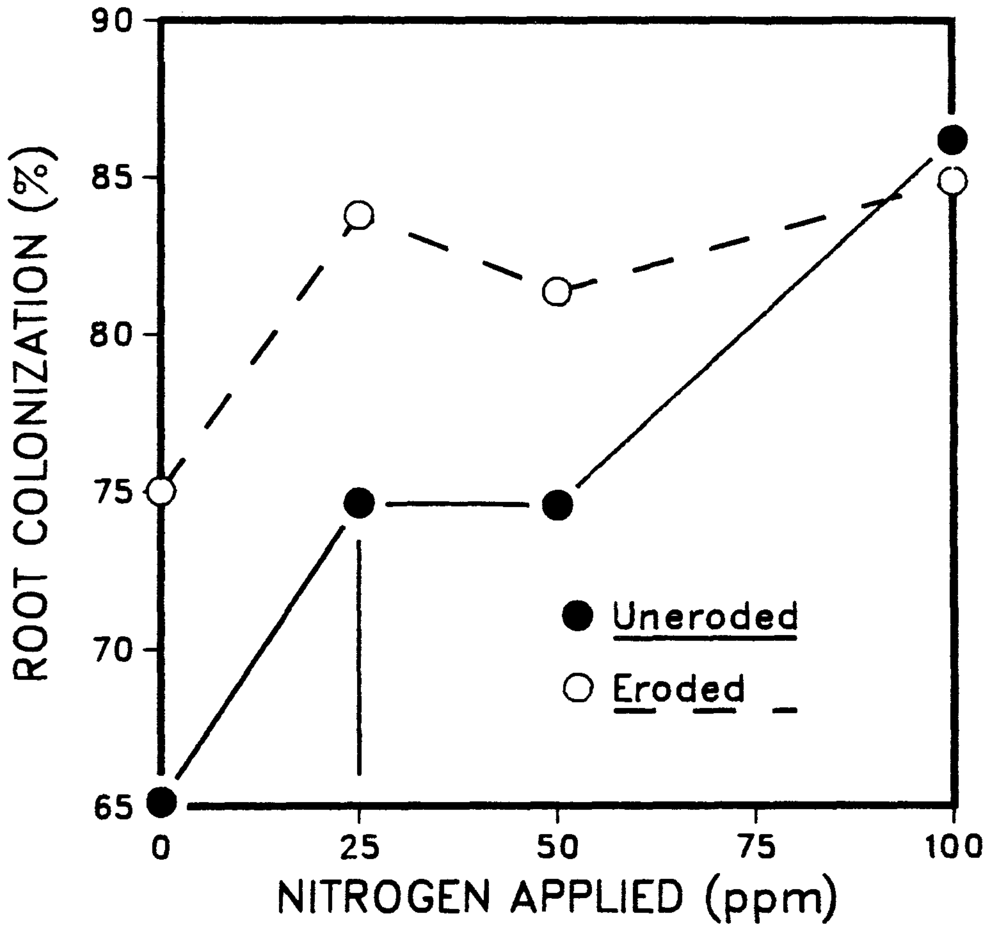

FIG. 7.1. The influence of inorganic N on the extent of VAM colonization of roots of cowpea grown in uneroded or eroded soil inoculated with G. aggregatum. Vertical bar represents LSD at the 5% level.

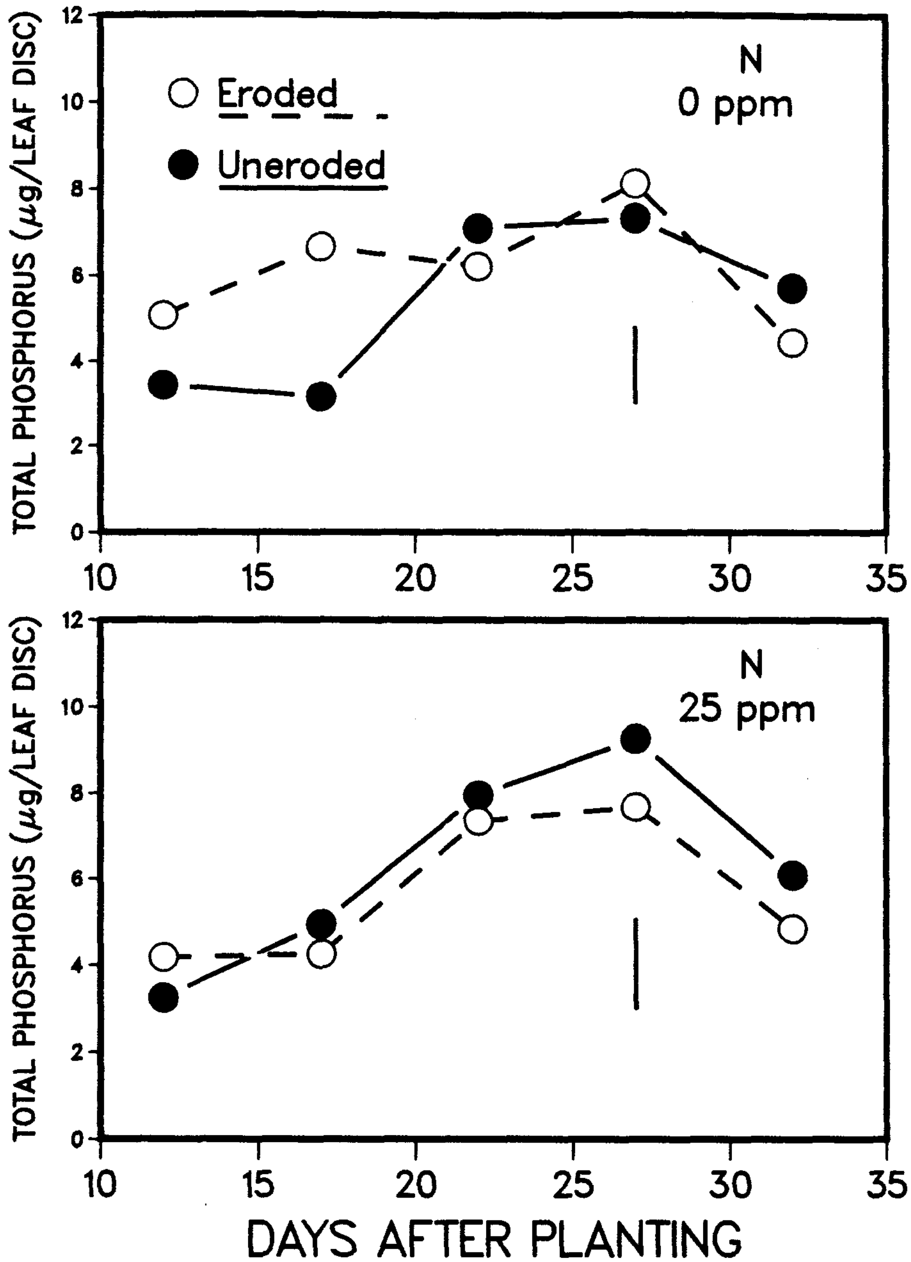

FIG. 7.2 The influence of inorganic N on the development of mycorrhizal effectiveness in cowpea grown in uneroded or eroded soil inoculated with G. aggregatum. Vertical bars represent LSD at the 5% level.

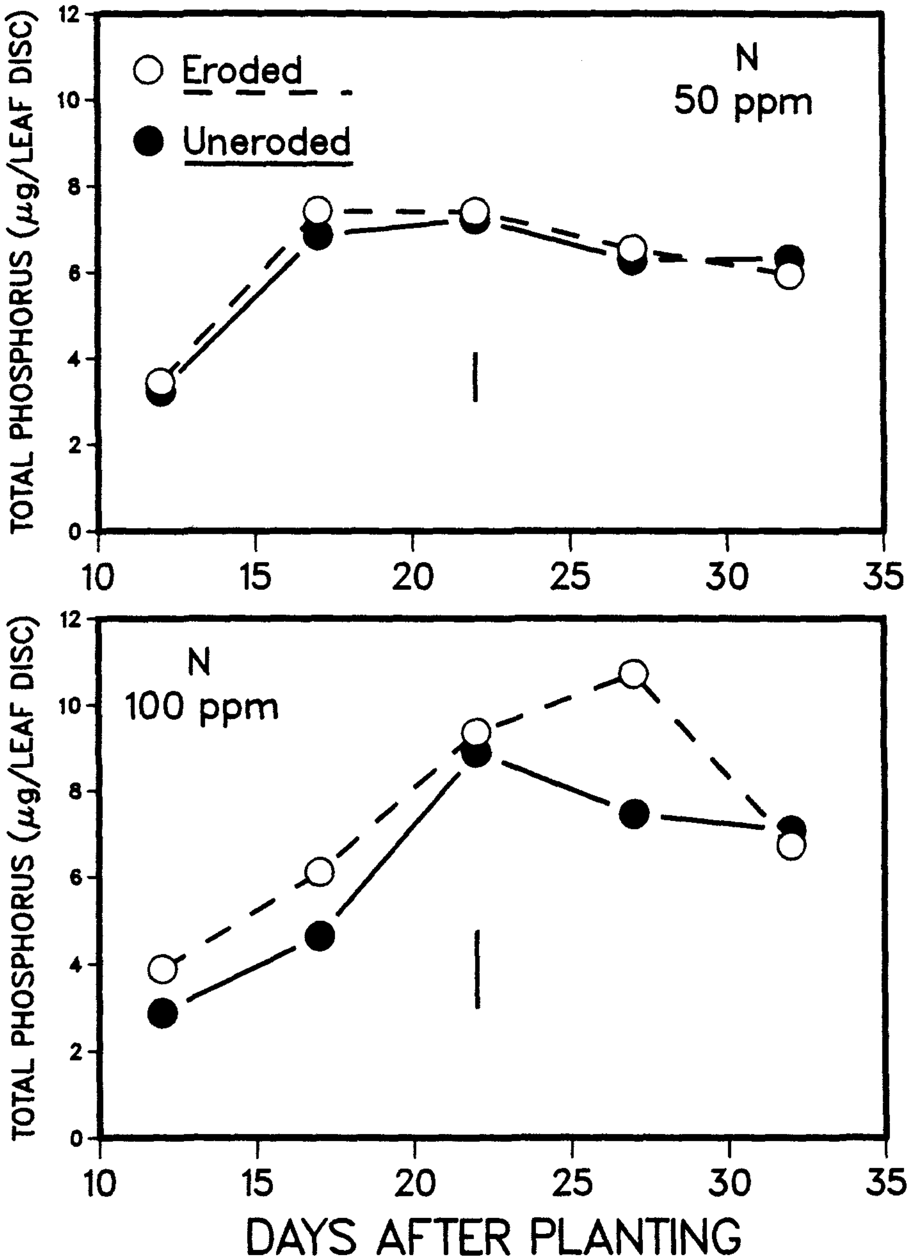

FIG. 7.2. Continuation.

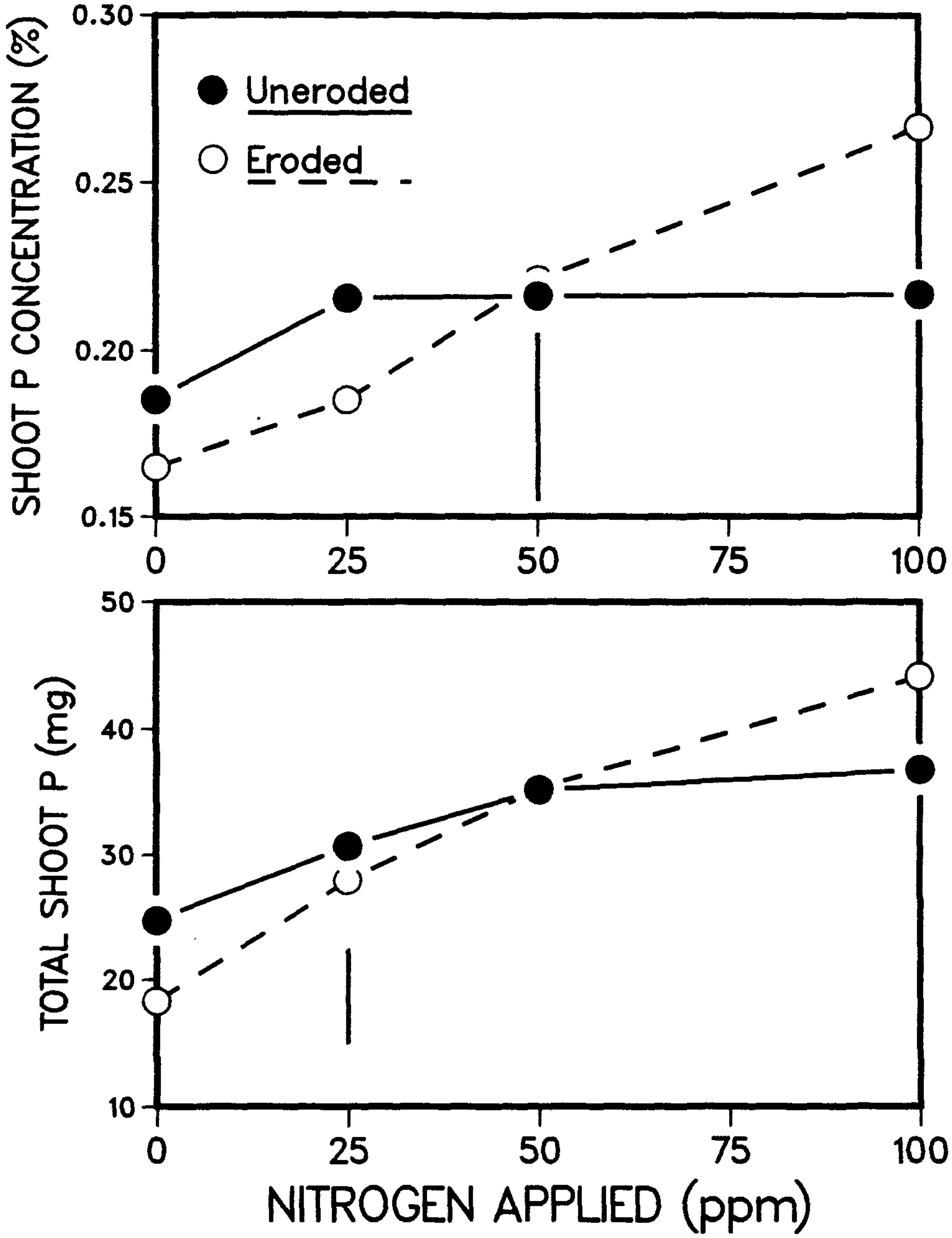

FIG. 7.3. The influence of inorganic N on shoot P status of cowpea grown in uneroded or eroded soil inoculated with G. *aggregatum*. Vertical bars represent LSD at the 5% level.

concentration with increase in inorganic N in the eroded soil while in the uneroded soil, shoot P concentration became stable after 25 ppm N. The trend in total shoot P was similar to that of shoot P concentration, except for the fact that in the uneroded soil, the total shoot P content of cowpea increased up to 50 ppm N (Fig. 7.3).

Figure 7.4 illustrates the influence of N on nodule dry weight of cowpea. Nodule dry matter production in cowpea was significantly lower in the eroded soil than in the uneroded soil when N was not added. With the addition of 25 ppm N, nodule dry weight in both the soil samples increased. At this level of N, nodule dry weight values in the two soil samples ceased to be significant. There was no further increase in nodule dry weight as the level of N was raised.

Shoot N concentration of cowpea increased significantly with the addition of the first level of N, but further increases in the level of N did not significantly influence tissue N concentration (Fig. 7.5). Nitrogen concentration in plants grown in the uneroded and eroded soils did not differ significantly from each other irrespective of N treatment. The trend of total shoot N content was similar to that of shoot N concentration except for the fact that in the uneroded soil the increase in shoot N content was almost linear upto 50 ppm while in the eroded soil the shoot N content levelled off after the addition of 25 ppm N (Fig. 7.5). As in shoot N concentration, there was no significant difference in shoot N content of cowpea grown in the eroded and uneroded soils.

Figure 7.6 illustrates the influence of N on shoot and root dry matter production of cowpea. Shoot dry matter production of cowpea

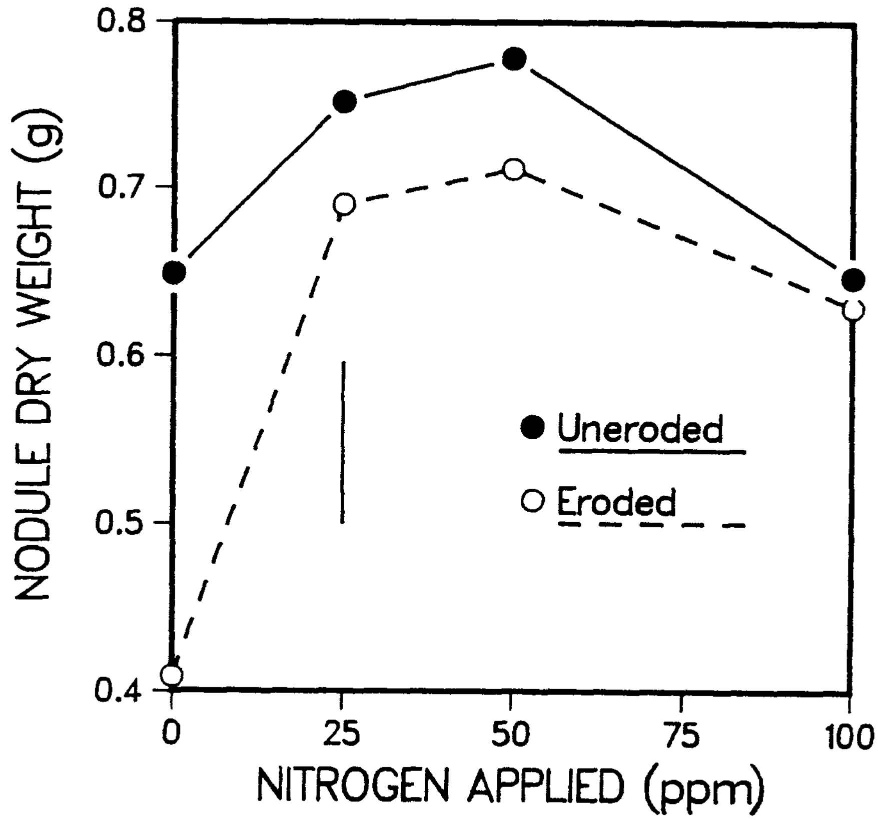

FIG. 7.4. The influence of inorganic N on nodule dry matter production of cowpea grown in uneroded or eroded soil inoculated with *G. aggregatum*. Vertical bar represents LSD at the 5% level.

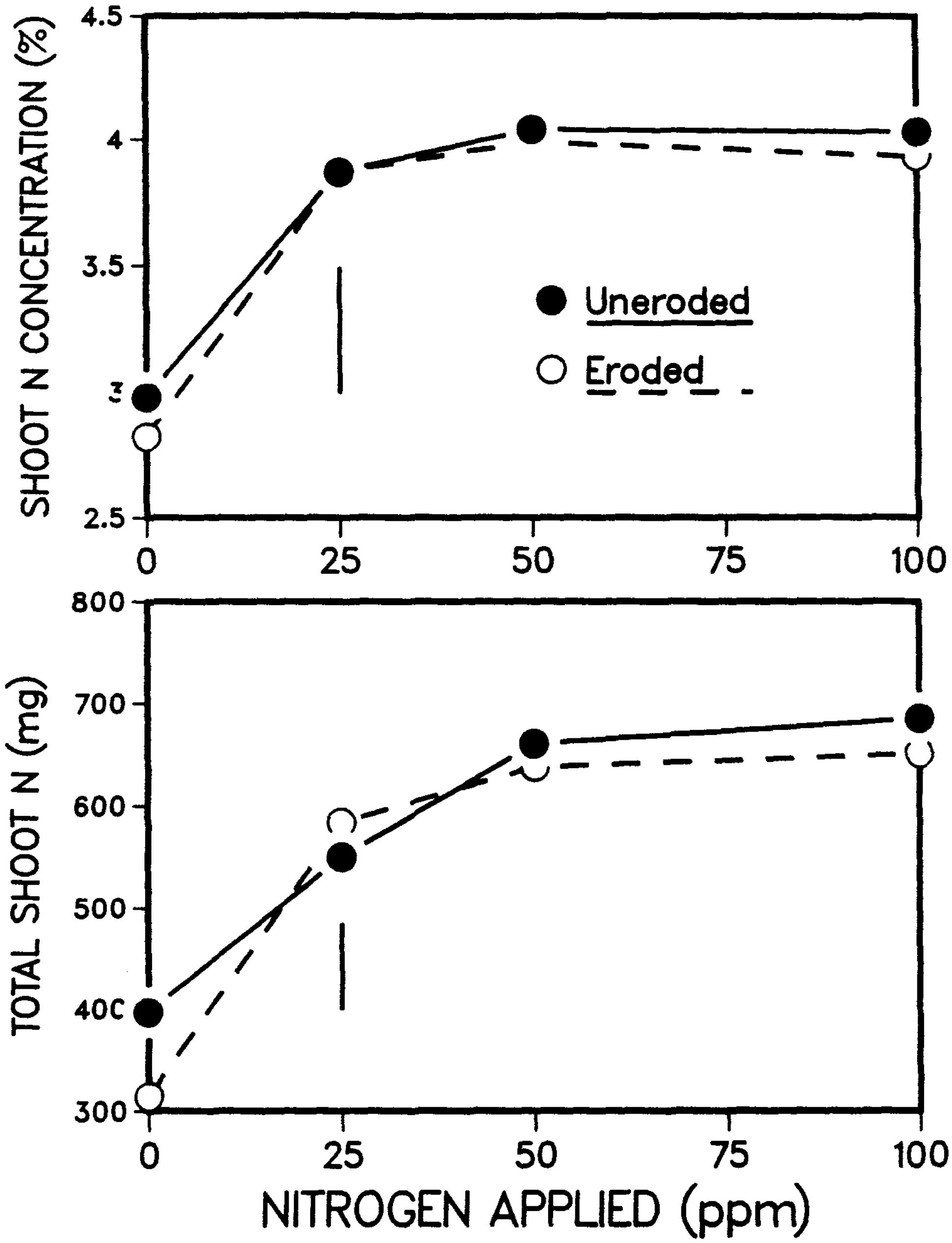

FIG. 7.5. The influence of inorganic N on shoot N status of cowpea grown in uneroded or eroded soil inoculated with G. aggregatum. Vertical bars represent LSD at the 5% level.

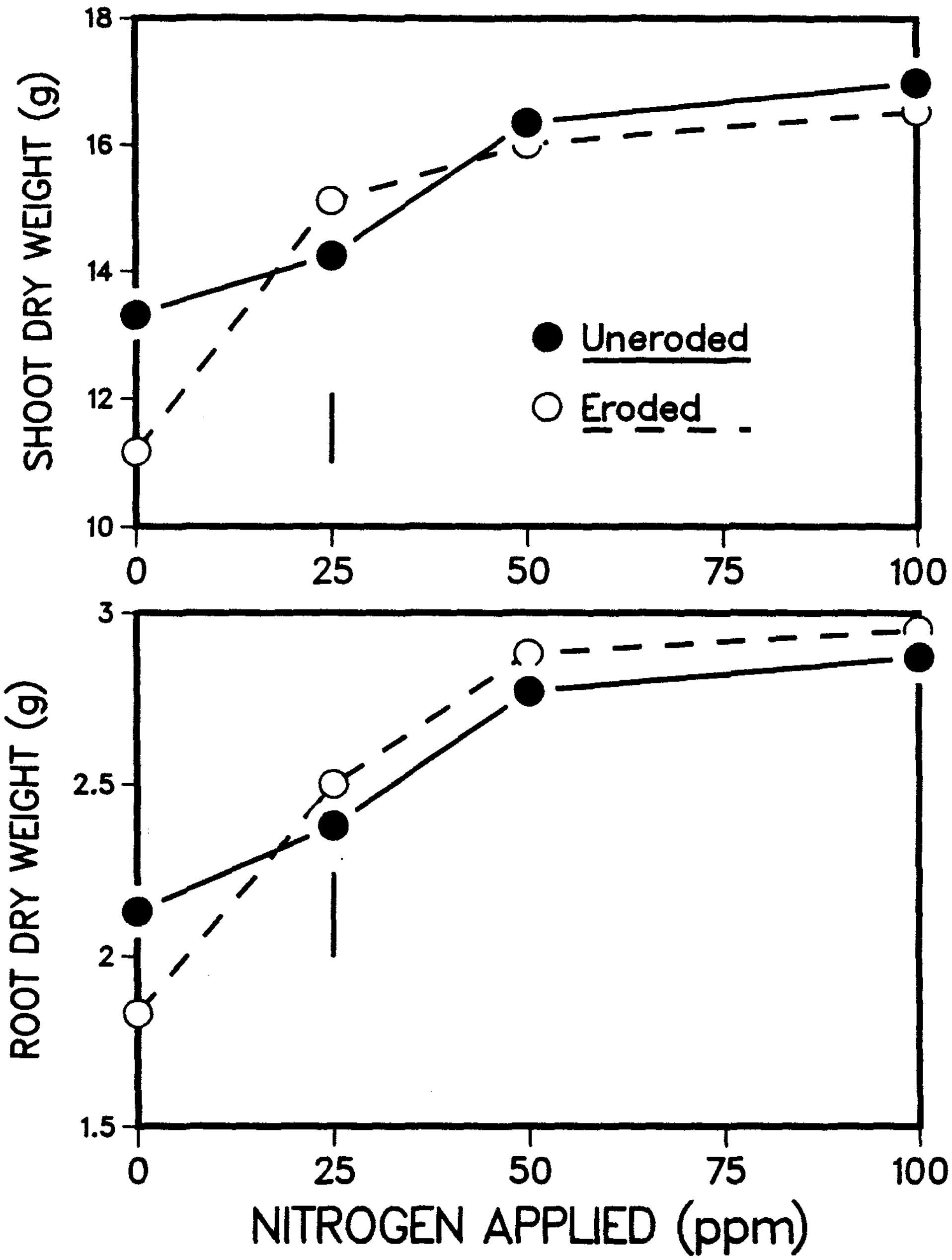

FIG. 7.6. The influence of inorganic N on dry matter production of cowpea grown in uneroded or eroded soil inoculated with G. aggregatum. Vertical bars represent LSD at the 5% level.

was significantly lower in the eroded soil than in the uneroded soil when N was not added. After the addition of 25 ppm N, however, there was a significant increase in shoot dry weight of cowpea in the eroded soil but not in the uneroded soil thereby removing the difference in shoot dry weight that was observed between the two soil samples in the absence of added N. There was, in general, an increasing trend in shoot dry weight above 25 ppm N but the shoot dry matter production in the two soil samples did not differ from each other. Trends in root dry matter data were similar to that observed in shoot dry matter data (Fig. 7.6). Root dry weight of cowpea was significantly lower in the eroded soil than in the uneroded soil in the absence of added N. By amending the soil samples with inorganic N at the rate of 25 ppm, the difference in root dry weight that existed between the two soil samples was removed. Maximum root growth was observed at 50 ppm N.

Leucaena. The extent of colonization of roots by G. aggregatum did not differ significantly in the eroded and uneroded soil in the absence of added N (Fig. 7.7). When N was added to the eroded soil at the rate of 25 ppm, there was a signifiacnt increase in the extent of root colonization. Above this level of N, there was no increase in root colonization. In the uneroded soil, on the other hand, infection level was significantly influenced only at the highest N level. The extent of colonization of roots was higher in the eroded soil than in the uneroded soil when N was applied to the soil samples at the rate of 25 and 50 ppm.

Mycorrhizal activity monitored in terms of the P content of subleaflets of leucaena grown in the eroded and uneroded soils amended

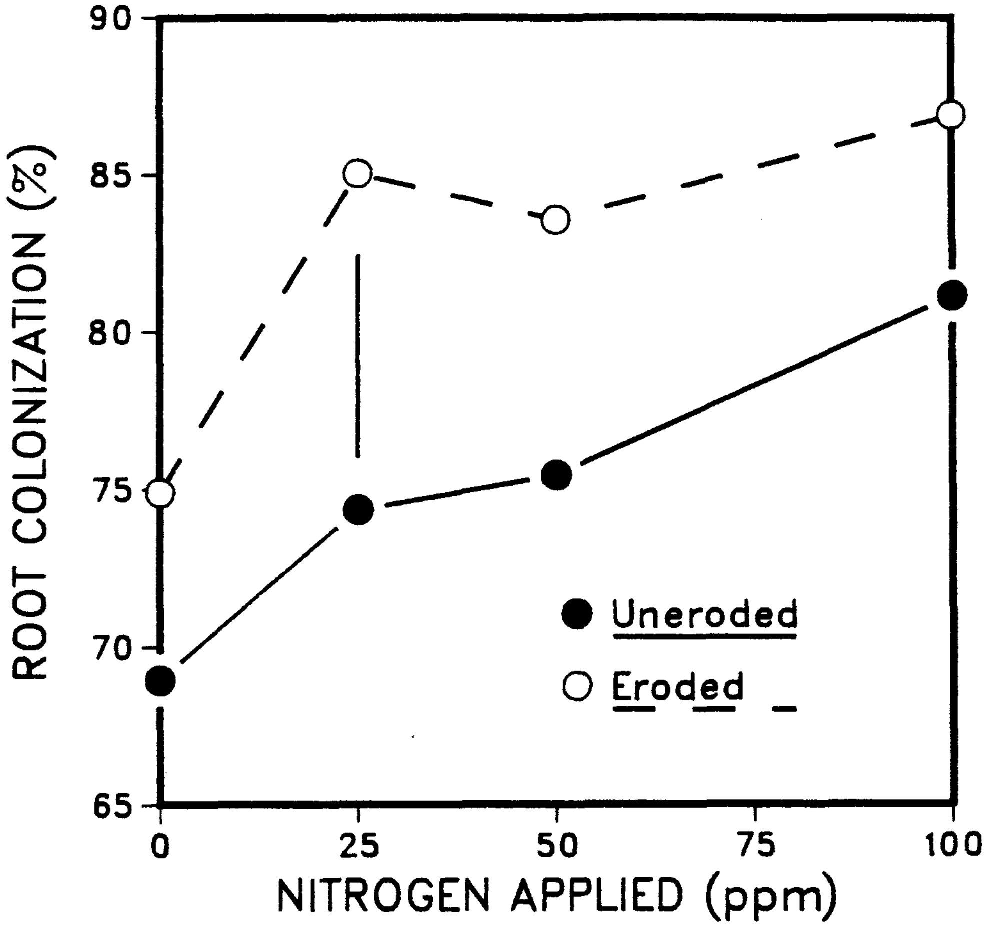

FIG. 7.7. The influence of inorganic N on the extent of VAM colonization of roots of leucaena grown in uneroded or eroded soil inoculated with G. aggregatum. Vertical bar represents LSD at the 5% level.

with different levels of inorganic N is shown in Fig. 7.8. In the absence of added N, mycorrhizal activity was lower in the eroded soil than in the uneroded soil. The activity increased begining on Day 17 and peaked at Day 22. The depression in activity that was observed in the eroded soil not amended with N disappeared when N was added to it at the rate of 25 ppm. Nitrogen application, in general, increased mycorrhizal activity in the eroded soil while it did not do so in the uneroded soil. When mycorrhizal activity was monitored in terms of the P concentration of subleaflets, similar results were obtained [Fig. C.6 (Appendix C)].

The application of N to the uneroded soil did not lead to significant increase in shoot P concentration of leucaena, while in the eroded soil, shoot P concentration increased when 100 ppm N was added to the soil (Fig. 7.9). Nevertheless, shoot P concentration of luecaena grown in the eroded and uneroded soils did not differ significantly from each other at any of the levels of N tested except at 100 ppm. Unlike shoot P concentration, total shoot P content was significantly lower in the eroded soil than in the uneroded soil in the absence of added N (Fig. 7.9). Total shoot P content increased with increase in N levels in the eroded soil but not in the uneroded soil. The initial difference in total shoot P content that was observed between the two soils ceased to be significant when N was added at the rate of 25 ppm.

Nodule dry matter production was similar in both soils when N was not added (Fig. 7.10). Nodule dry weight increased as a function of

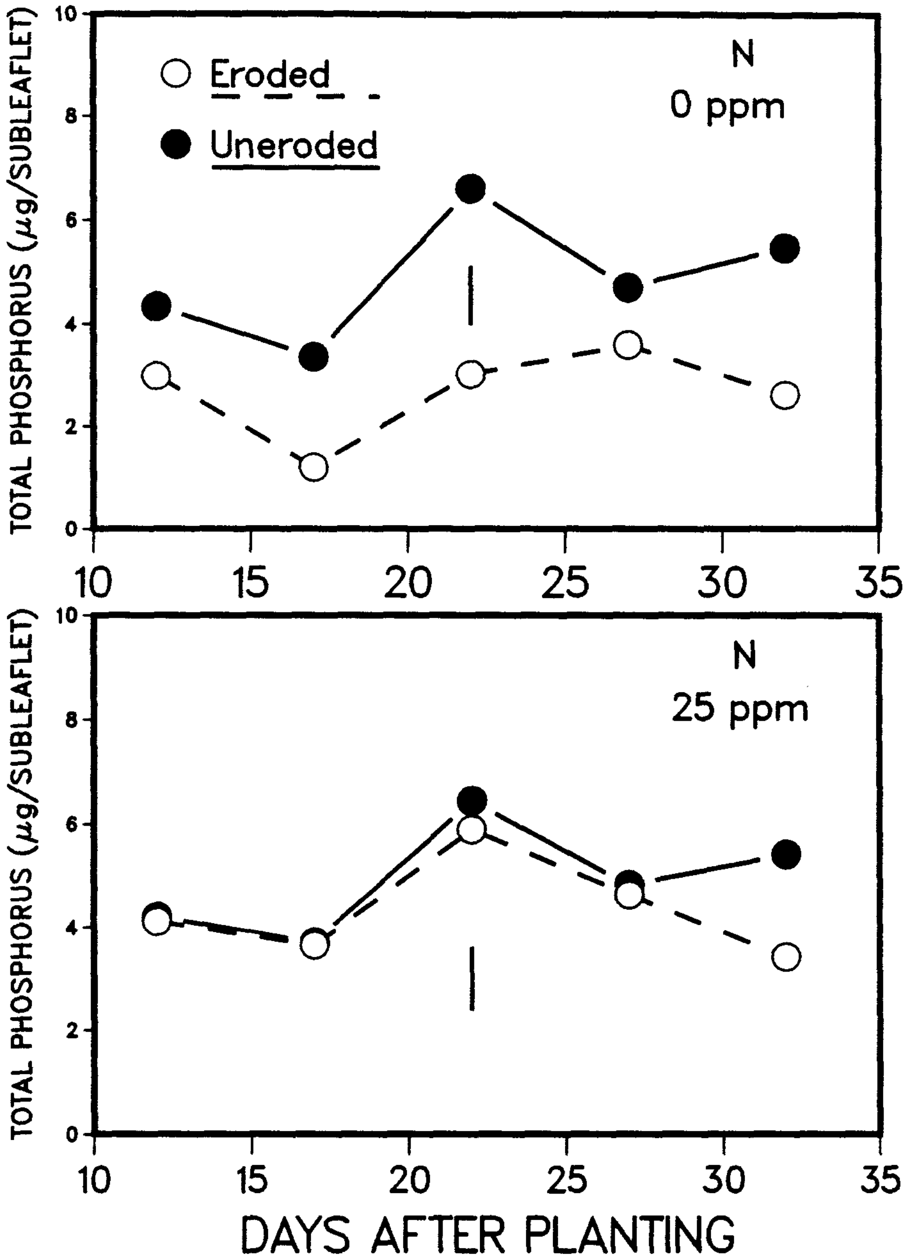

FIG. 7.8. The influence of inorganic N on the development of mycorrhizal effectiveness in leucaena grown in uneroded or eroded soil inoculated with G. aggregatum. Vertical bars represent LSD at the 5% level.

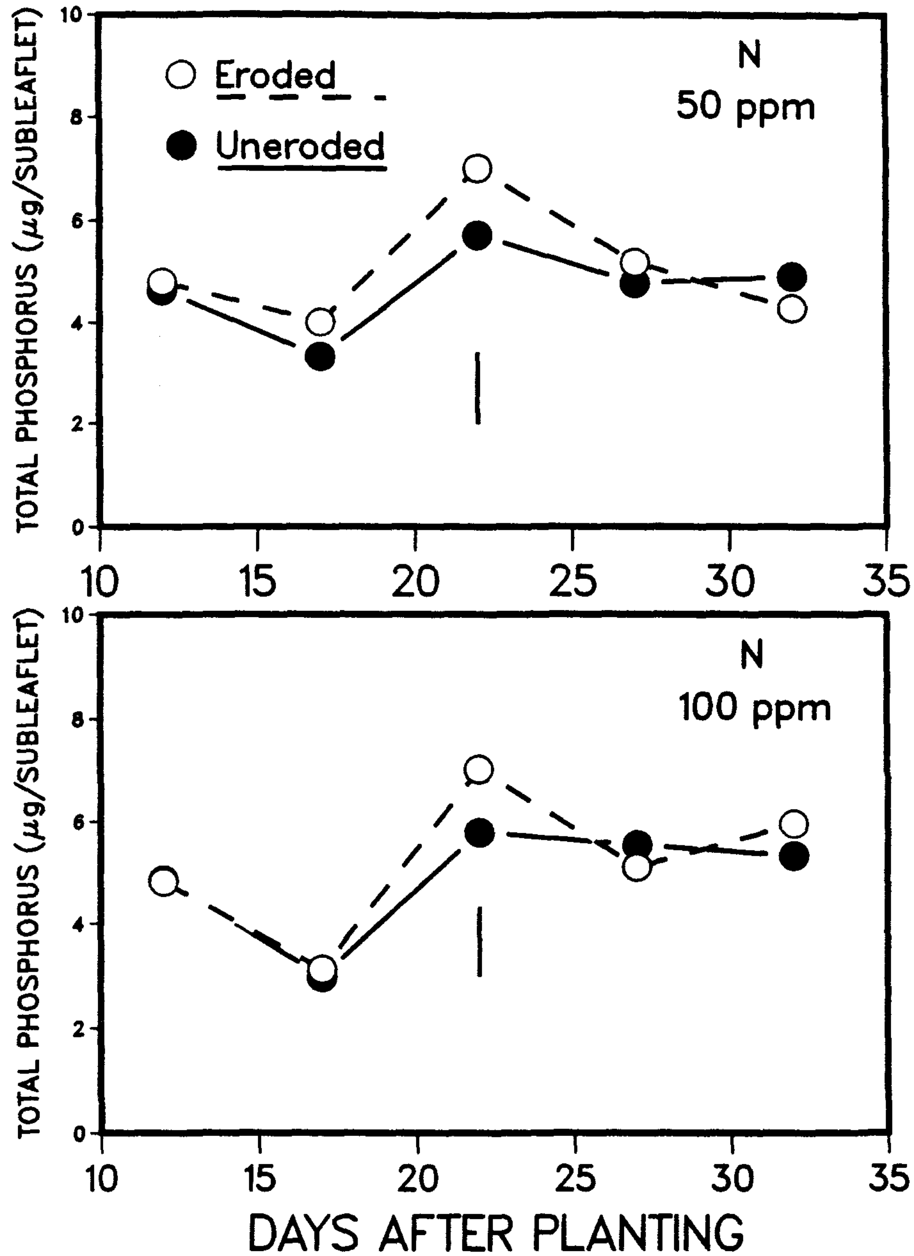

FIG. 7.8. Continuation.

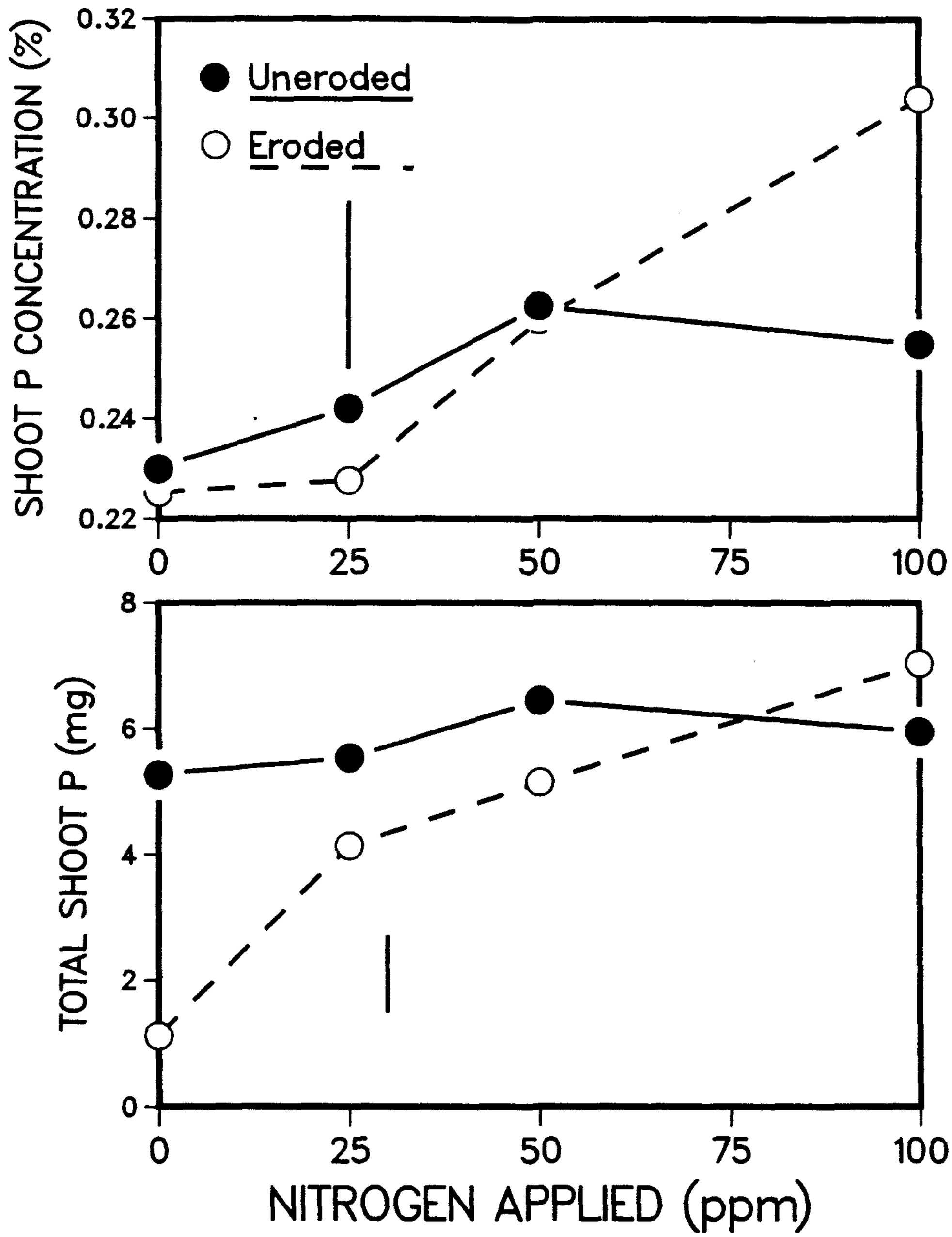

FIG. 7.9. The influence of inorganic N on shoot P status of leucaena grown in uneroded or eroded soil inoculated with G. aggregatum. Vertical bars represent LSD at the 5% level.

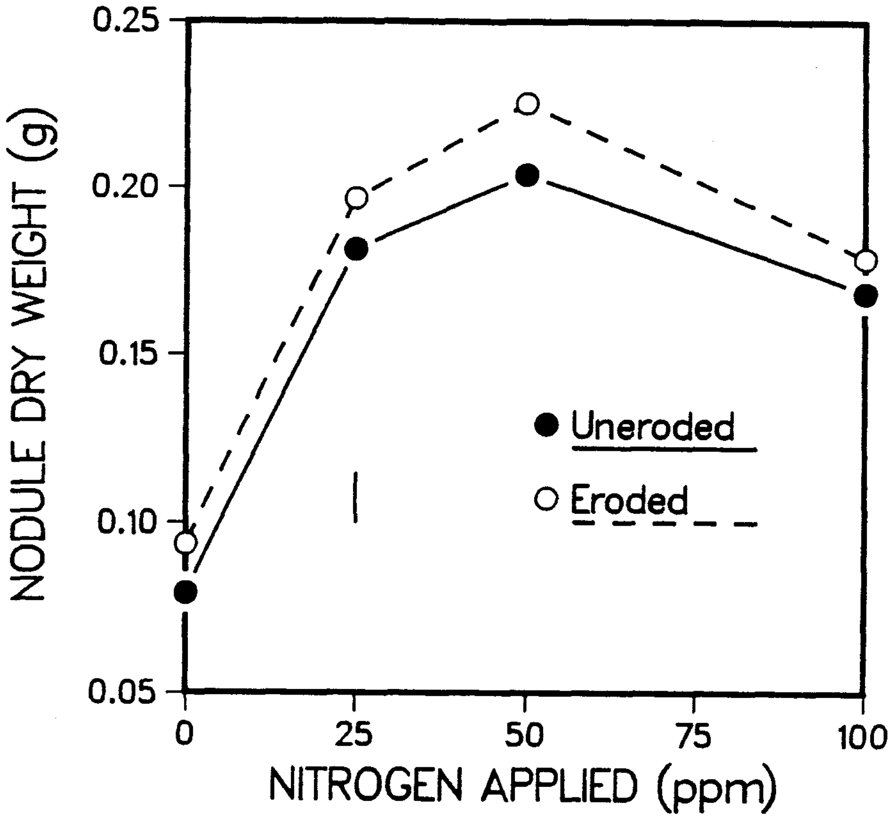

FIG. 7.10. The influence of inorganic N on nodule dry matter production of leucaena grown in uneroded or eroded soil inoculated with G. aggregatum. Vertical bar represents LSD at the 5% level.

increases in the level of N added, reaching a maximum value at 50 ppm N. Above 50 ppm N, nodule dry matter production decreased.

Shoot N concentrations of leucaena grown in the eroded and uneroded soils were similar in the absence of added N (Fig. 7.11). The shoot N concentration in both soils increased as level of added N was increased, reaching a maximum value at 50 ppm N. Above this level of N, shoot N concentration did not change. Nitrogen concentration in plants grown in the eroded and uneroded soils did not differ significantly from each other irrespective of N treatments. The total shoot N content of leucaena was lower in the eroded soil than in the uneroded soil when N was not added to the soil samples (Fig. 7.11). The first level of N amendment led to a significant increase in total shoot N content in the eroded soil but not in the uneroded soil, thereby eliminating the difference in total shoot N content that was observed between the two soil samples initially. In general, there was an increasing trend in total shoot N content of leucaena above 25 ppm N but the two soil samples did not significantly differ from each other.

The effects of N application on shoot and root dry matter production of leucaena are depicted in Fig. 7.12. Shoot and root dry weights were significantly lower in the eroded soil than in the uneroded soil in the absence of added N. When N was added at the rate of 25 ppm, shoot and root dry weights increased significantly in the eroded soil while there was no change in the uneroded soil. The difference in shoot and root dry matter yields that was observed between the two soil samples in the absence of added N, disappeared

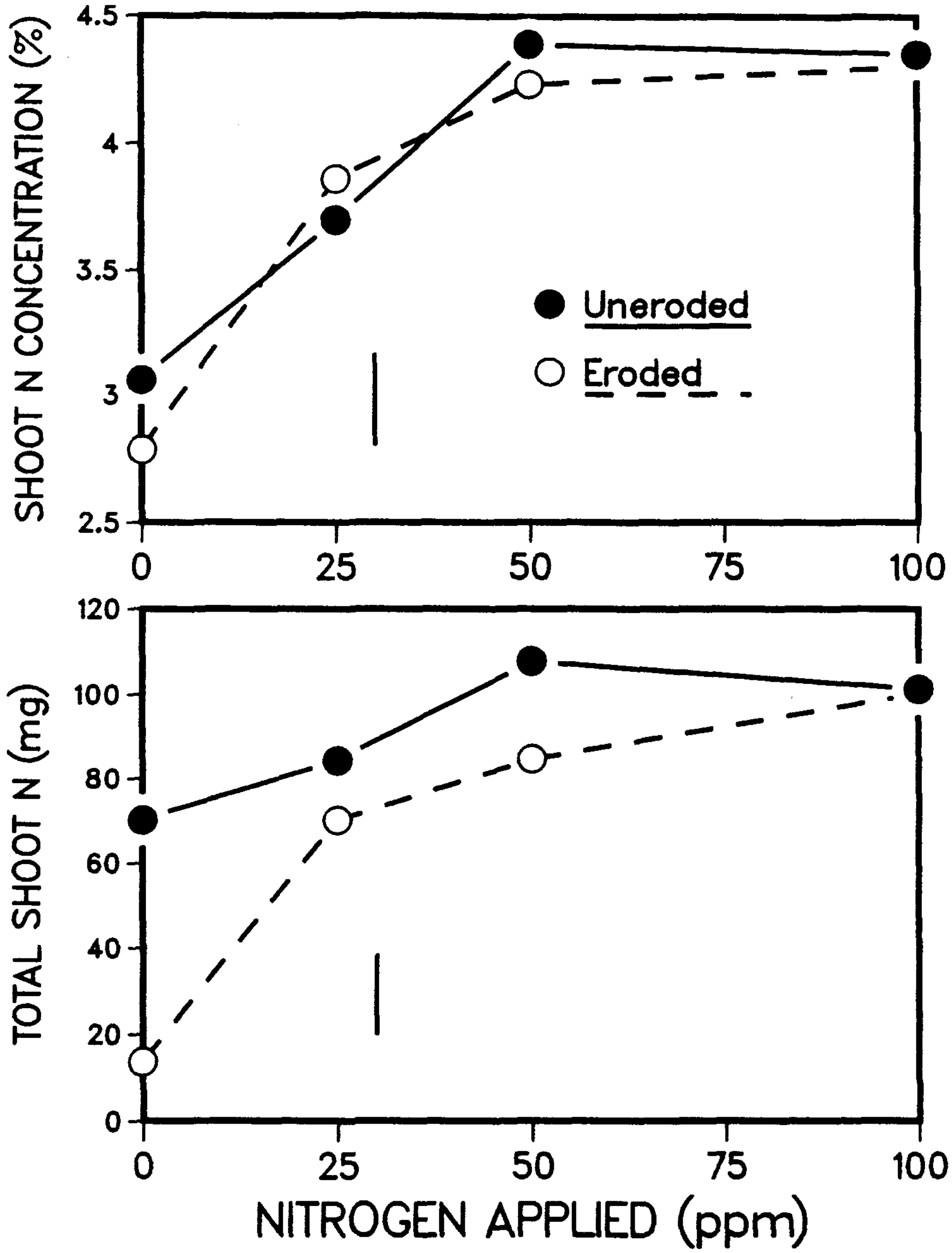

FIG. 7.11. The influence of inorganic N on shoot N status of leucaena grown in uneroded or eroded soil inoculated with <u>G. aggregatum</u>. Vertical bars represent LSD at the 5% level.

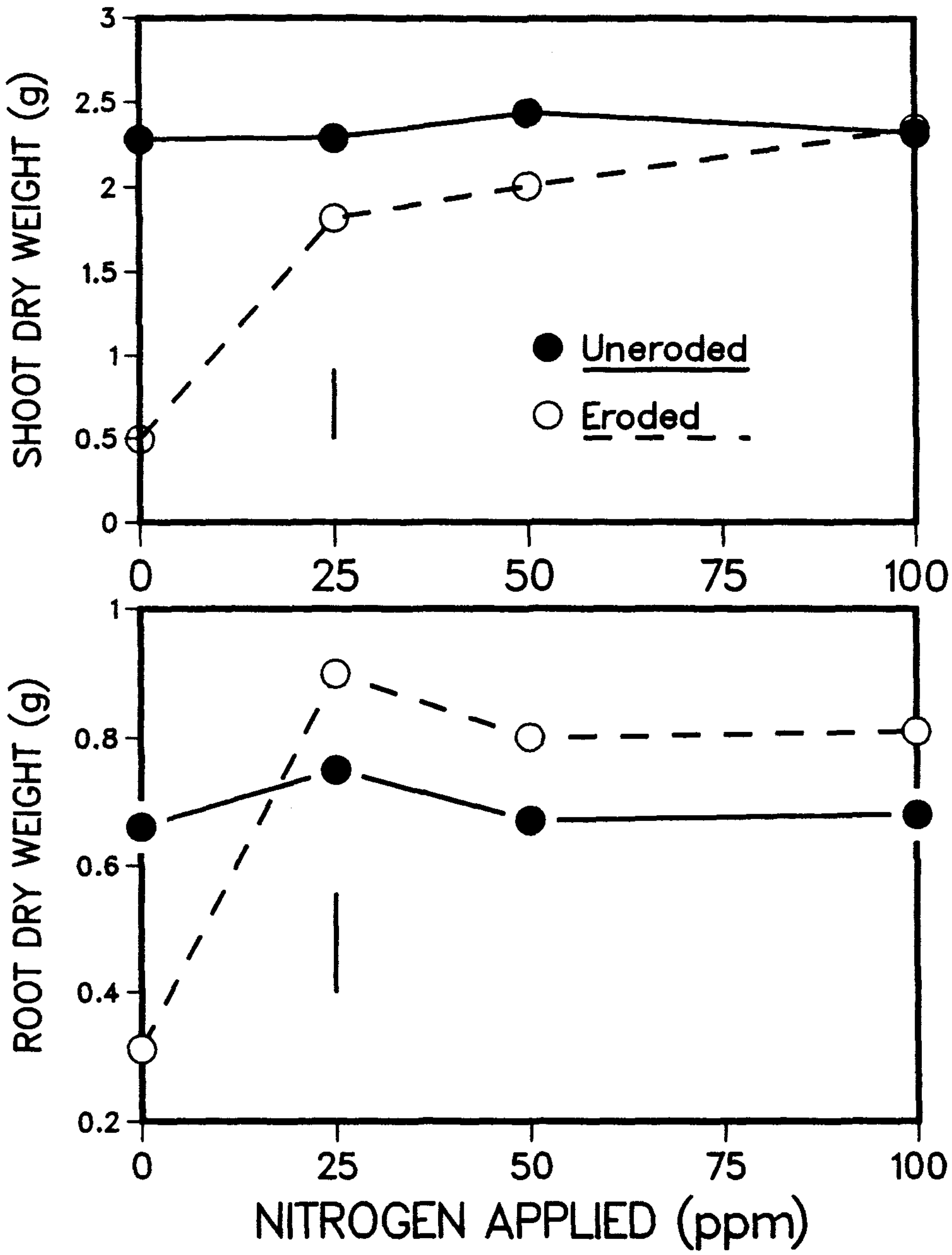

FIG. 7.12. The influence of inorganic N on dry matter production of leucaena grown in uneroded or eroded soil inoculated with _G. aggregatum_. Vertical bars represent LSD at the 5% level.

when N was added at the rate of 25 ppm. A further increase in N level increased shoot dry weight only in the eroded soil, but the increase was significant only after the addition of 100 ppm of N. Nevertheless, shoot and root weight values observed in the eroded and uneroded soil samples were not significantly different from each other.

DISCUSSION

Decreased mycorrhizal activity (effectivity) observed in leucaena when grown in the eroded soil in the absence of added N seems to be a result of N deficiency because of 2 reasons. Firstly, a decrease in activity was not observed in the uneroded soil which had a higher N content than the former soil, and secondly, the depression in VAM activity observed in the eroded soil was removed when N was added to the soil. It appears from the results that N influenced mycorrhizal activity only at the initial level of N application, i.e., 25 ppm. This level of inorganic N could, thus, be defined as the optimum level for mycorrhizal activity in the Wahiawa soil. Although the difference in mycorrhizal activity in the unamended eroded and uneroded soils disappeared when N was added at the rate of 25 ppm or more, the extent of colonization of roots was high in the eroded soil than in the uneroded soil. The higher level of colonization of roots by VAM fungi in the eroded soil is, probably, due to low population of indigenous VAM fungi and other microorganisms in that soil. This indicates that the infectivity of VAM fungi does not necessarily correspond to the effectivity. The effectivity measured in terms of leaf P status was similar to the effectivity measured in terms of other parameters such as shoot P uptake and dry matter yields. In cowpea, the effect of N on mycorrhizal activity was not clear. The low mycorrhizal activity observed in the uneroded soil in the absence of added N was ultimately increased in about 3 weeks. This initial depression in mycorrhizal activity observed in the unamended uneroded soil may be a result of sampling error because other measurements of effectivity (shoot P uptake and dry matter yields) do not show this depression. It appears from these results that N application to soil samples did not appreciably influence mycorrhizal activity in cowpea. However, application of N did increase the extent of root colonization by VAM fungi, shoot P uptake and dry matter yields. On the basis of root colonization data it appears that colonization of roots by VAM fungi is quite sensitive to N deficiency. One common thing that was observed in both plant species is the fact that there was an increasing trend in root colonization with increases in the level of N.

Stimulation of root colonization by VAM fungi, as a result of N application observed in this study, was not in agreement to the findings of others (3,14,20). Chambers et al. (3) observed a significant reduction in root colonization of cowpea as a result of ammonium-N application when the soil was inoculated with naturally occuring endophytes. They argued that assimilation of NH_4^+ by plant roots resulted in a drop in pH in soil rhizosphere [because of the release of H^+ as a result of NH_4^+ assimilation (15)]. The authors believe that lowering of pH might have affected the introduced endophyte adversely in the rhizoshphere. The disagreement of my results from the above can be explained by the fact that I used ammonium nitrate as the source of N which has both NH_4^+ and NO_3^- forms of N. Nitrate-N has been shown to be less deleterious to VAM colonization than NH_4-N (3). On the other hand, Brown et al. (2) observed increases in the percentages of mycorrhizal roots and in intensities of root segment infection at N level that produced maximum growth of sweetgum seedlings. The level of infection was, however, reduced as the rate of N application was increased above the level needed for maximum plant growth. Similarly, Hepper (16) observed that mycorrhizal infection increased as the level of nitrate was increased' but the extent of colonization of root depended on the level of P applied. She hypothesized that the ratio of nitrate to phosphate in the plant nutrient solution is important in determining mycorrhizal infection and a ratio of above 15 would be needed to achieve a reasonable level of infection. Such a hypothesis, however, may have little application in soil because many nutrients when applied to soil are adsorbed or lost and the ratio of nutrients will vary.

The difference in trend of shoot N content of cowpea observed in the eroded and uneroded soil is, probably, a reflection of shoot dry matter yield. Cowpea seems to differ from leucaena in terms of N requirement for maximum shoot and root dry matter production. Application of 50 ppm N was necessary to achieve maximum dry matter yields in cowpea. In leucaena, on the other hand, application of 25 ppm N was required for maximum plant growth in the eroded soil while no N was needed in the uneroded soil. The higher N requirement of cowpea is, probably, due to its higher rate of dry matter accumulation compared to that of leucaena. The maximum shoot and root dry matter yields of cowpea at harvest was about 7 and 4 times higher, respectively, than that of leucaena. Furthermore, the two leguminous species may differ in potential nitrogen fixation rates (8,11).

The reduction in nodule dry weight observed in cowpea when grown in the eroded soil compared to that in the uneroded soil when N was not added indicates that the N status of the eroded soil was not adequate for optimum nodulation. The fact that nodule dry weight in the eroded soil increased upon the addition of N is suggestive of the fact that the starter N requirement of cowpea was satisfied. In leucaena, on the other hand, there was no difference in nodule dry matter production between the two soil samples in the absence of added N. This difference in nodulation between the two plant species is, probably, related to the differences in the rate of initiation of N_2-fixation. Host factors are believed to affect nodule initiation in legumes (13). Increase in nodule dry weight with N application upto a certain level and then inhibition at a higher level observed in this study is in agreement with previous findings (5,9,10,17,18,21). The effects of nitrogen fertilizers on nodulation and N_2-fixation by legumes are complex and depend largely on the level of nitrogen in soil. The situation may be further complicated by the rapid change in the nitrate and ammonium levels in soil because one form may be more deleterious than the other. Application of starter N can promote nodulation by overcoming N deficiency during the establishment of the nitrogen fixation process (12). On the other hand, the inhibition of nodulation by high levels of inorganic N, may be due to their effect on lectins, or specific recognition glycoproteins, on the root hair surface. Combined N is believed to interfere with the binding of rhizobia to plant lectins on the root hairs (6,7). Combined N also affects N_2-fixation by the repression of nitrogenase synthesis (23) and by converting leghemoglobin into an inactive form (22).

The results of this study indicate the potential beneficial effect of N application on the growth of mycorrhizal cowpea and leucaena in eroded soils. The growth depression that is observed in the eroded soil due to N deficiency can, thus, be overcome by adding N. The results also indicate the existence of optimum levels of starter N for root colonization and nodulation of mycorrhizal cowpea and leucaena. So, N should also be applied along with other nutrients in order to establish mycorrhizal legumes in eroded soils.

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CHAPTER 8

OPTIMIZATION OF CHEMICAL INPUTS FOR ESTABLISHING EFFECTIVELY NODULATED AND MYCORRHIZAL COWPEA AND LEUCAENA IN AN ERODED SOIL

INTRODUCTION

The level of nutrients in soil is often an important factor determining mycorrhizal activity. Habte and Manjunath recently (16) showed that soil available phosphorus is very crucial for mycorrhizal activity, and there appears to be a level at which mycorrhizal activity is optimum. Nitrogen and phosphorus are also believed to interact with each other in influencing mycorrhizal activity (6). Similarly soil pH also affects endophytes and there is an optimum pH range for each mycorrhizal species (18).

Soil erosion is a serious problem in many parts of the world (8). Erosional loss of soil is often associated with significant loss of nutrients such as P, N, Ca, Mg etc. (see appendix A) and reduction in the population and activity of VAM fungi (see Chapter 3). In previous experiments (Chapter 4), my attempt to rehabilitate soils subjected to simulated erosion by inoculating with VAM fungi was not successful presumably because of the deficiency of nutrients. The results of experiments subsequently conducted to determine the influence of individual nutrient amendments on the VAM symbiosis (Experiments 5, 6 and 7) showed that the symbiosis was enhanced by the application of P, lime and inorganic N at optimum amounts.

The objective of this study was to determine the influence of combining these nutrients at optimal levels on mycorrhizal activity and growth of nodulated cowpea and leucaena in an eroded soil.

MATERIALS AND METHODS

Cowpea or leucaena was grown in the eroded or uneroded soil with one of three nutrient categories in the presence or absence of VAM inoculation. The nutrient categories were: (1) complete set of nutrients consisting of phosphorus, lime, nitrogen, potassium, magnesium, sulfur, zinc, copper and boron (complete); (2) basal nutrients consisting of potassium, magnesium, sulfur, zinc, copper and boron (basal); and (3) no nutrients added (none). For the complete set of nutrients, P was added to get a soil solution level of 0.026 mg/l, soil samples were limed to obtain a pH value of 6.0 and inorganic N was added at the rate of 50 ppm. The rate of application of basal nutrients were the same as outlined in Chapter 2.

Cowpea or leucaena was grown in the soils for 32 and 37 days, respectively. Every 5 days the P contents of leaf discs of cowpea or subleaflets of leucaena were determined. After harvest, the extent of colonization of roots by VAM fungi, shoot, root and nodule dry weights, and concentrations of P, N, Cu and Zn in shoot were determined.

RESULTS

Cowpea. The extent of colonization of cowpea roots by VAM fungi in the eroded and uneroded soils amended with nutrients is illustrated in Fig. 8.1. Root colonization in the eroded soil not inoculated with *G. aggregatum* did not change significantly in response to nutritional amendments. Inoculating the soil with *G. aggregatum* increased the extent of root colonization significantly. The extent of colonization, however, was greatest when cowpea was grown in the inoculated soil in the presence of all the nutrients. The results were similar in the uneroded soil. The extent of colonization in the uneroded soil was higher than that in the eroded soil except when the soils were inoculated with *G. aggregatum* and amended with all the nutrients (complete).

Mycorrhizal activity monitored by determining the P content of leaf discs of cowpea grown in the eroded and uneroded soils amended with different nutrient categories are shown in Figures 8.2 and 8.3. Mycorrhizal activity in the eroded soil not inoculated with *G. aggregatum* did not change significantly in response to nutritional amendments (Fig. 8.2). However, when the soil was inoculated with *G. aggregatum* in the presence of all the nutrients (complete), there was a significant increase in mycorrhizal activity which began on Day 17 and peaked on Day 27. At this time, the activity was about 7 times higher than that observed in the soil not amended with nutrients. Inoculation of the soil amended with only basal nutrients did not significantly improve mycorrhizal activity. Mycorrhizal activity in

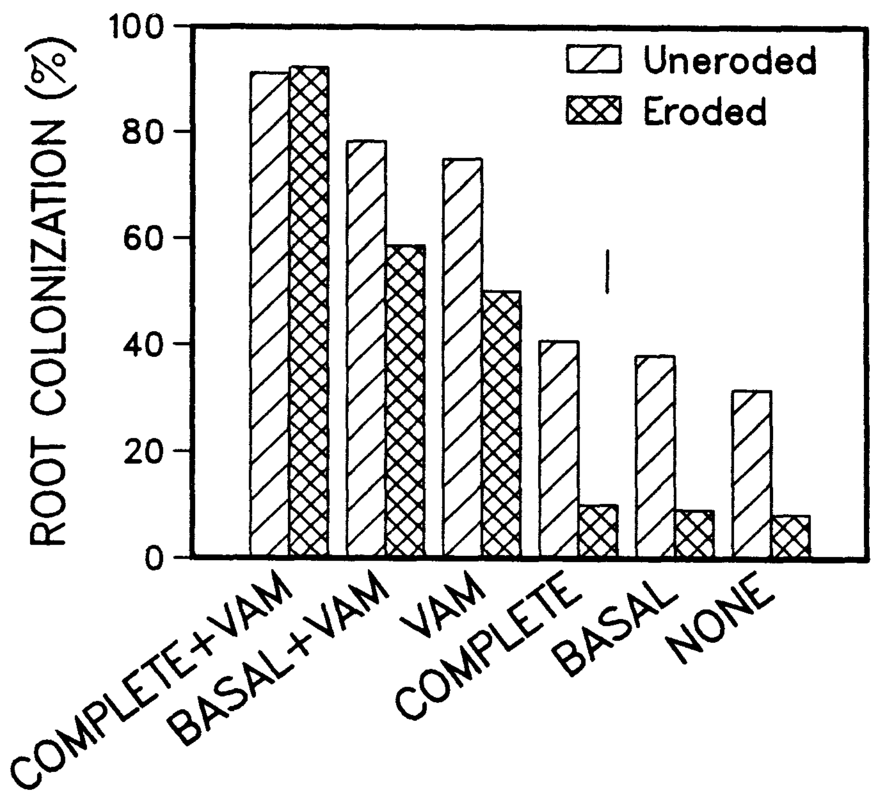

FIG. 8.1. The influence of nutrient amendments and VAM inoculation on the extent of colonization of roots of cowpea grown in uneroded or eroded soil. Vertical bar represents LSD at the 5% level.

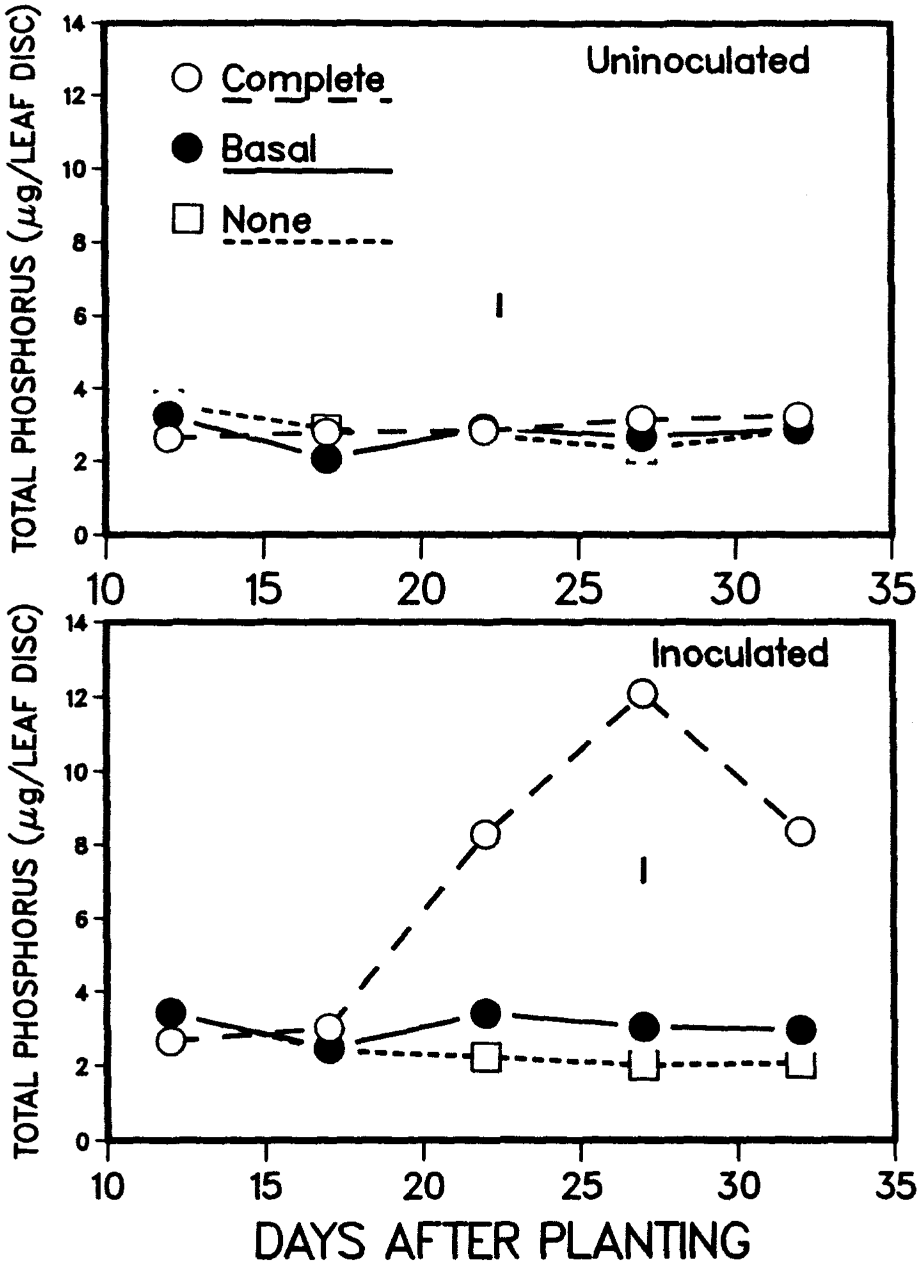

FIG. 8.2. The influence of nutrient amendments and VAM inoculation on the development of mycorrhizal effectiveness in cowpea grown in eroded soil. Vertical bars represent LSD at the 5% level.

the uneroded soil not inoculated with Glomus aggregatum also did not change significantly in response to nutritional amendments (Fig. 8.3). However, when the soil was inoculated with G. aggregatum in the presence of all the nutrients (complete), there was a significant increase in mycorrhizal activity which began on Day 22 and peaked on Day 27. At the peak period, the activity was about 4-5 times higher than that observed in the soil not amended with nutrients. When the inoculated soil was amended only with basal nutrients, mycorrhizal activity did not change significantly compared to that observed in the soil not amended with nutrients. Similar trends were observed when mycorrhizal activity was monitored in terms of the P concentration of leaf discs [Figures B.8 and B.9 (Appendix B)].

Influence of nutrient amendments on shoot P concentration and shoot P content of cowpea is illustrated in Fig. 8.4. In the absence of G. aggregatum, amendement of nutrients in the eroded soil had no influence on shoot P concentration while in the uneroded soil, shoot P concentration was increased in the presence of all nutrient (complete). On the other hand, when the soil samples were inoculated with G. aggregatum, there were significant increases in shoot P concentration of cowpea due to the addition of basal or complete set of nutrients in both eroded and uneroded soils. The highest shoot P concentration, nevertheless, was observed when cowpea was grown in the inoculated soil samples amended with all the nutrients (complete). Shoot P concentrations of cowpea grown in the eroded and uneroded soils were similar except when grown in the uninoculated soil amended with all the nutrients. The total shoot P content of cowpea grown in

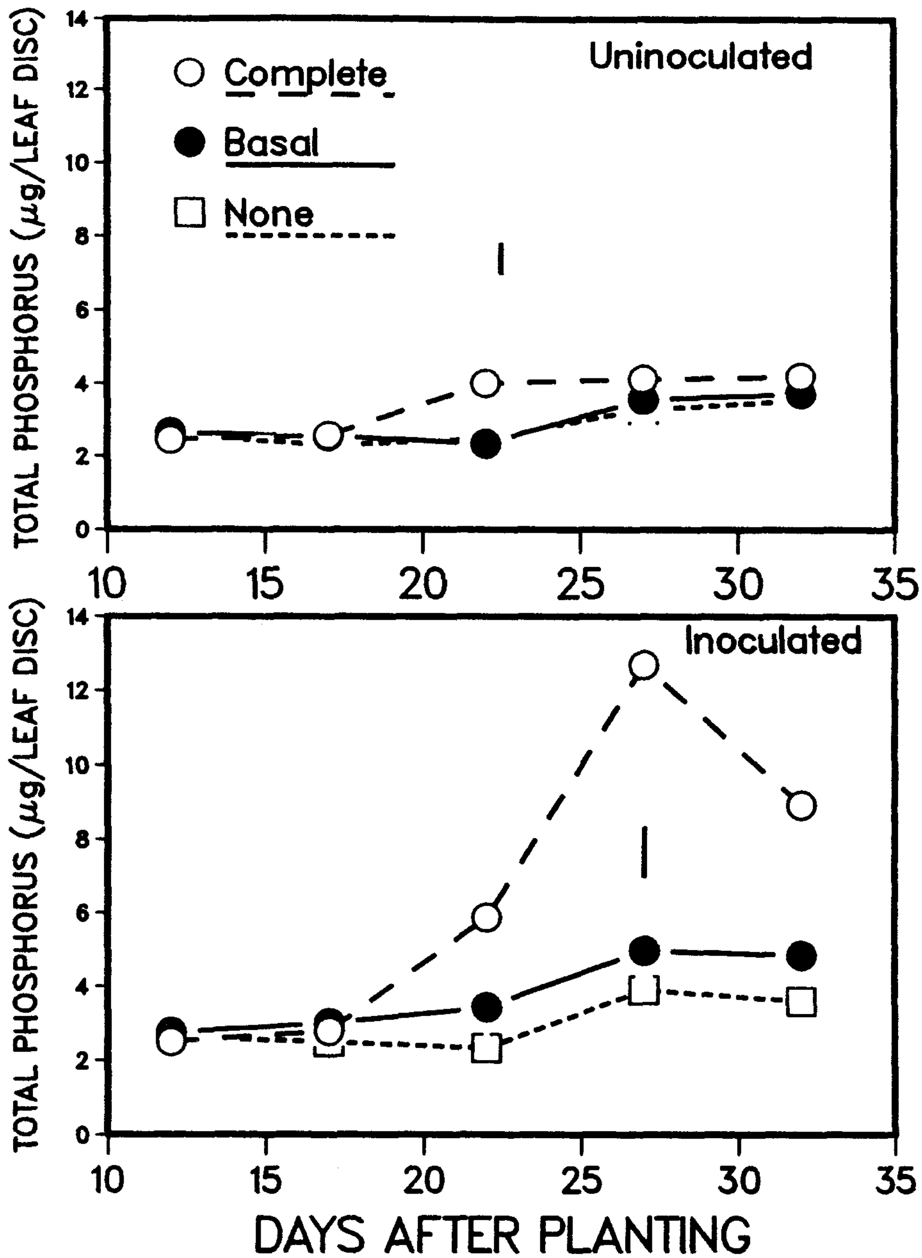

FIG. 8.3. The influence of nutrient amendments and VAM inoculation on the development of mycorrhizal effectiveness in cowpea grown in uneroded soil. Vertical bars represent LSD at the 5% level.

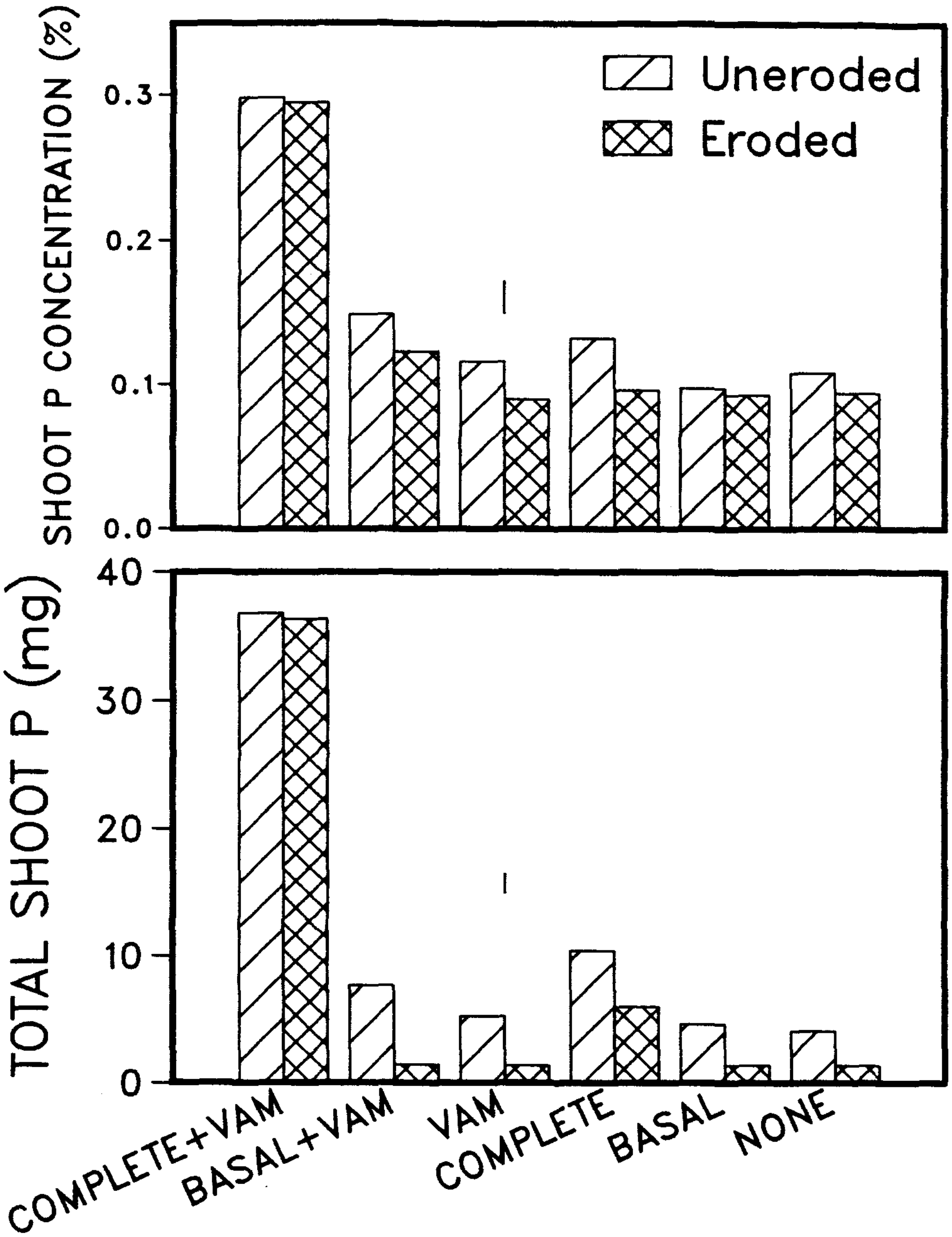

FIG. 8.4. The influence of nutrient amendments and VAM inoculation on shoot P status of cowpea grown in uneroded or eroded soil. Vertical bars represent LSD at the 5% level.

the absence of G. aggregatum increased significantly when the soil samples were amended with all the nutrients (complete). In the presence of the fungus, the shoot P content of cowpea grown in the eroded soil increased when amended with all the nutrients while in the uneroded soil, the shoot P content increased when the soil was amended with basal nutrients or the complete set of nutrients. The shoot P content of cowpea was lower in the eroded soil than in the uneroded soil in all the treatments except when grown in the presence of G. aggregatum and all the nutrients.

Shoot Cu concentration of cowpea grown in the absence of G. aggregatum was significantly increased when all the nutrients (complete) were added to the eroded soil but not to the uneroded soil (Fig. 8.5). On the other hand, when cowpea was grown in the presence of G. aggregatum, there was an increase in shoot Cu concentration in both the eroded and uneroded soils when amended with all the nutrients. The two soil samples, however, did not differ from each other in terms of shoot Cu concnetration. The total shoot Cu content of cowpea increased significantly when the soil samples were amended with all the nutrients in the presence or absence of G. aggregatum. The highest shoot Cu content was observed when cowpea was grown in the inoculated soil samples amended with all the nutrients.

Shoot Zn concentration of cowpea increased only when the soil sampels were inoculated with G. aggregatum and amended with all the nutrients (Fig. 8.6). The two soil samples did not differ from each other in terms of shoot Zn concentration. Shoot Zn content, however, increased in both the inoculated and uninoculated soil samples when

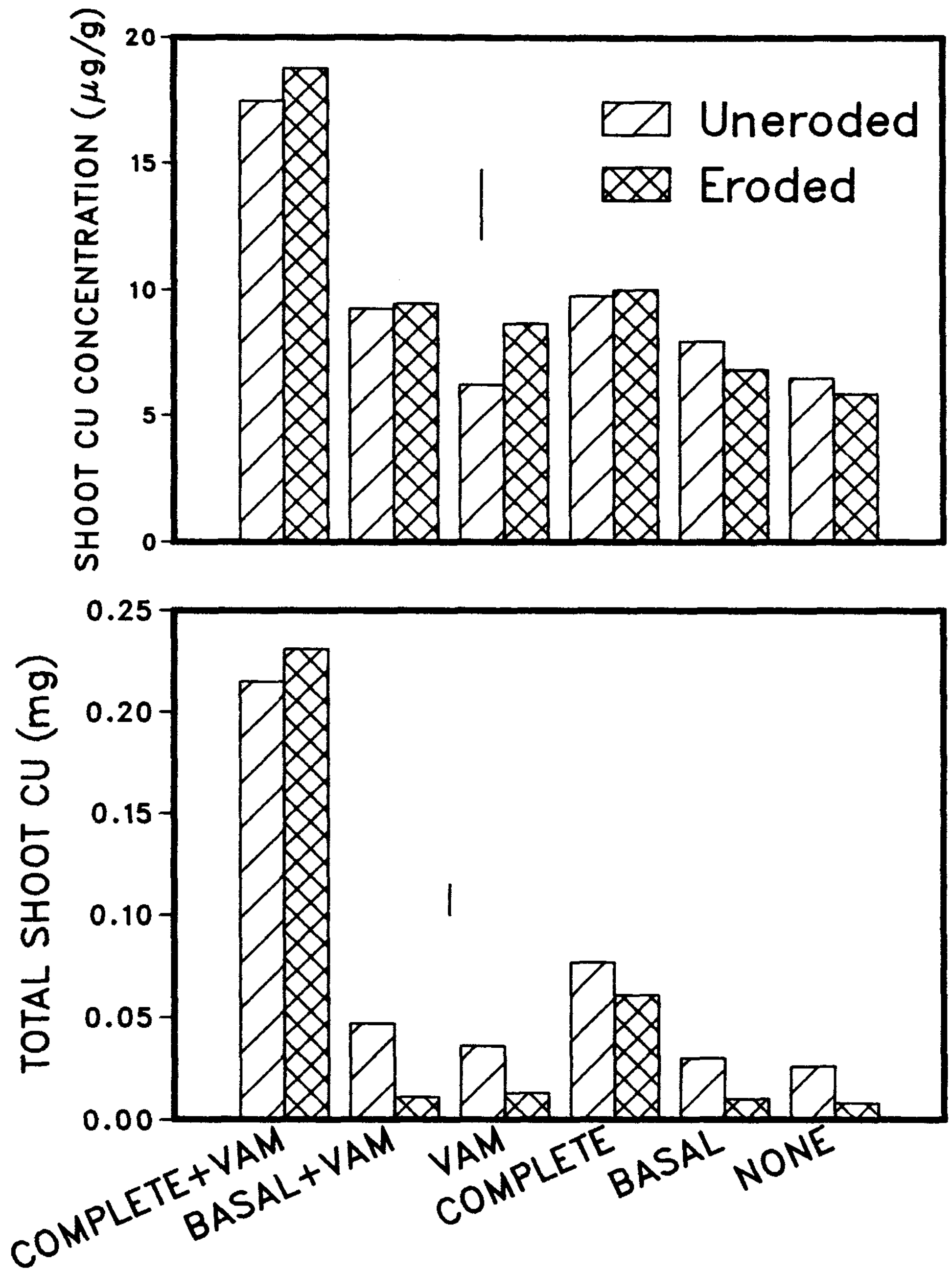

FIG. 8.5. The influence of nutrient amendments and VAM inoculation on shoot Cu status of cowpea grown in uneroded or eroded soil. Vertical bars represent LSD at the 5% level.

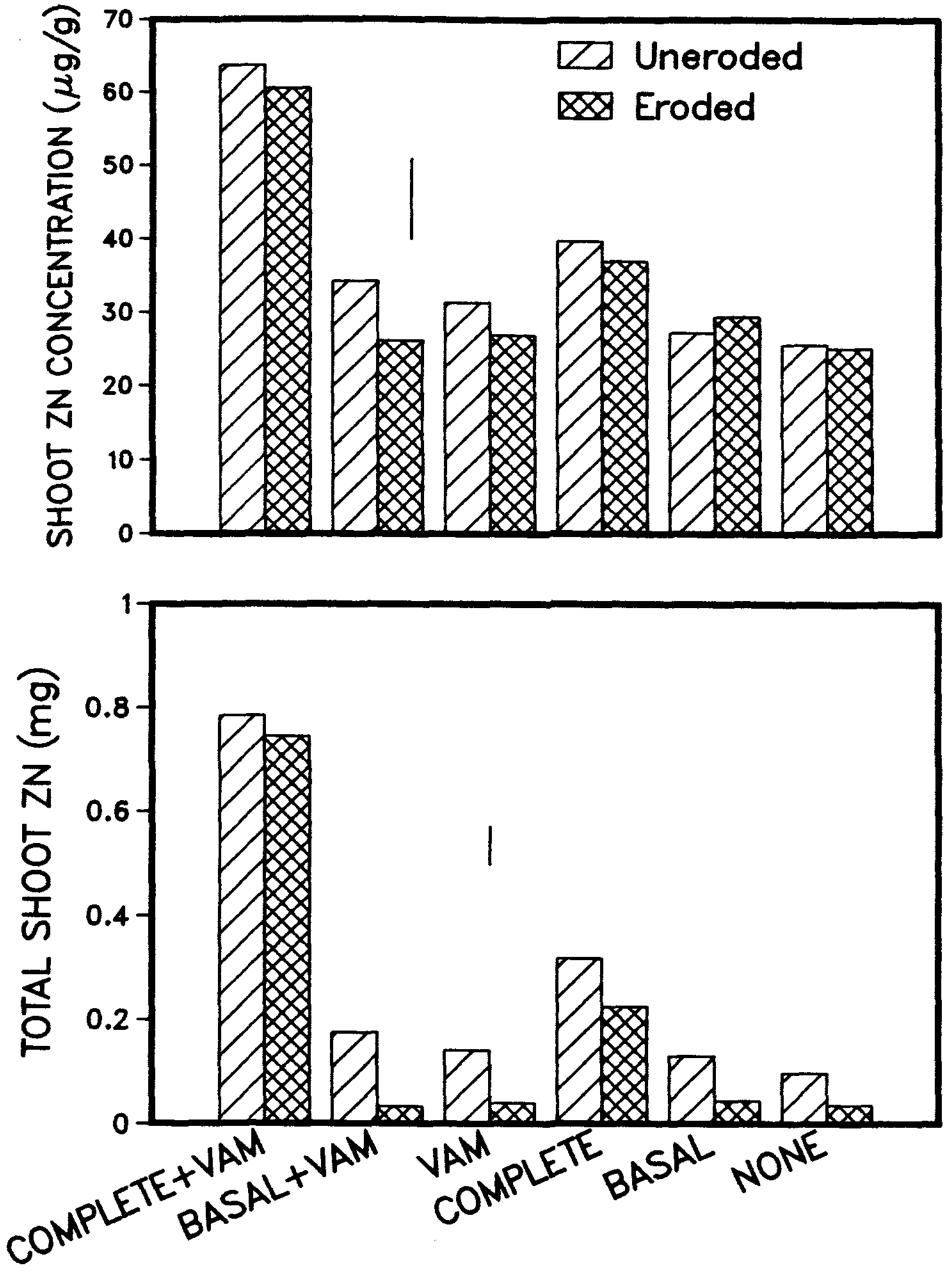

FIG. 8.6. The influence of nutrient amendments and VAM inoculation on shoot Zn status of cowpea grown in uneroded or eroded soil. Vertical bars represent LSD at the 5% level.

the soils were amended with all the nutrients. The highest shoot Zn content was observed when cowpea was grown in the inoculated soil samples amended with all the nutrients (Fig. 8.6).

Nodule dry matter production of cowpea increased significantly only when the soil samples were inoculated with G. aggregatum and amended with all the nutrients (complete) (Fig. 8.7). Shoot N concentration of cowpea also increased significantly only when the soil samples were amended with all the nutrients, which increased further on inoculation with G. aggregatum (Fig. 8.8). Maximum shoot N concentration was observed when cowpea was grown in the inoculated soil samples amended with all the nutrients. Shoot N concentration of plants grown in eroded and uneroded soils did not differ from each other. Like shoot N concentration, the shoot N content of cowpea also significantly increased when grown in soil amended with all the nutrients in the presence or absence of G. aggregatum (Fig. 8.8). However, cowpea grown in the inoculated soil samples amended with all the nutrients had the highest shoot N content. The shoot N content of cowpea was significantly lower in the eroded soil than in the uneroded soil when amended with basal nutrients alone or when not amended with nutrients in the presence or absence of G. aggregatum.

Shoot dry matter production of cowpea grown in the eroded soil increased significantly when amended with all the nutrients, which increased further on inoculation with G. aggregatum (Fig. 8.9). Shoot dry weight of cowpea was greatest when grown in the presence of G. aggregatum and all the nutrients. Similar was the trend of results observed in the uneroded soil. Shoot dry matter production of cowpea

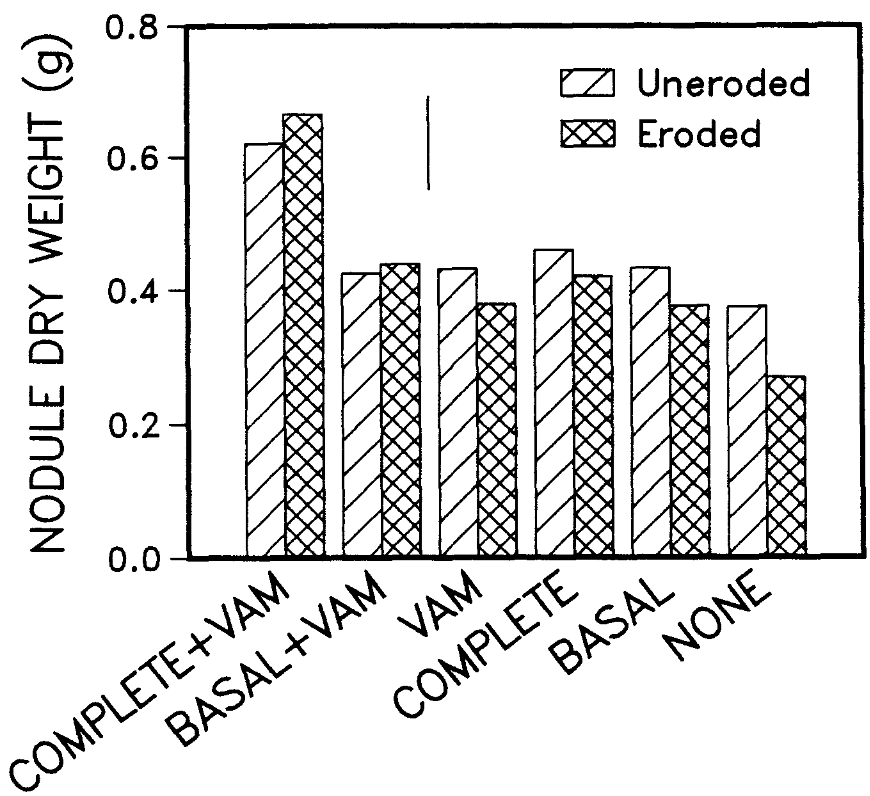

FIG. 8.7. The influence of nutrient amendments and VAM inoculation on nodule dry matter production of cowpea grown in uneroded or eroded soil. Vertical bar represents LSD at the 5% level.

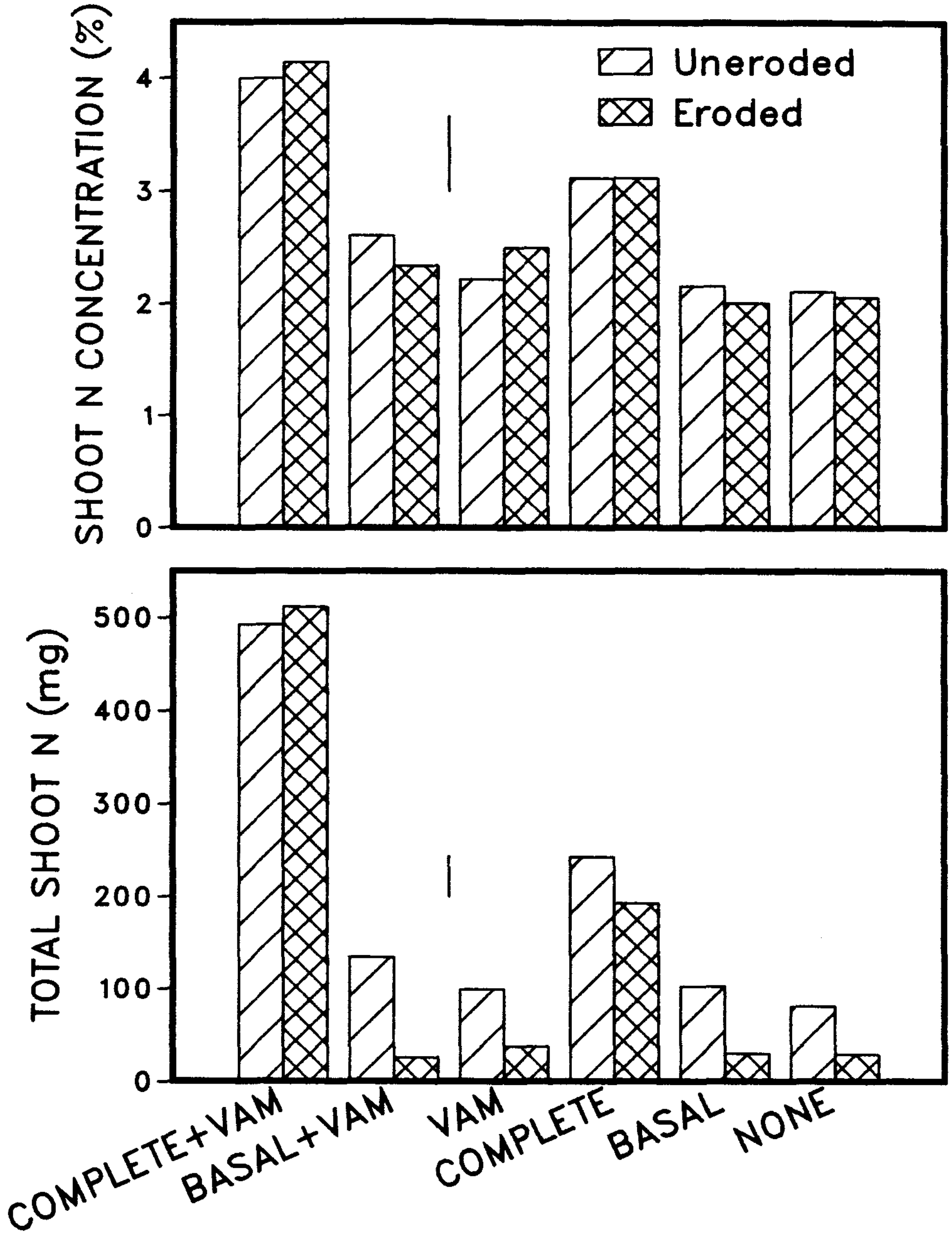

FIG. 8.8. The influence of nutrient amendments and VAM inoculation on shoot N status of cowpea grown in uneroded or eroded soil. Vertical bars represent LSD at the 5% level.

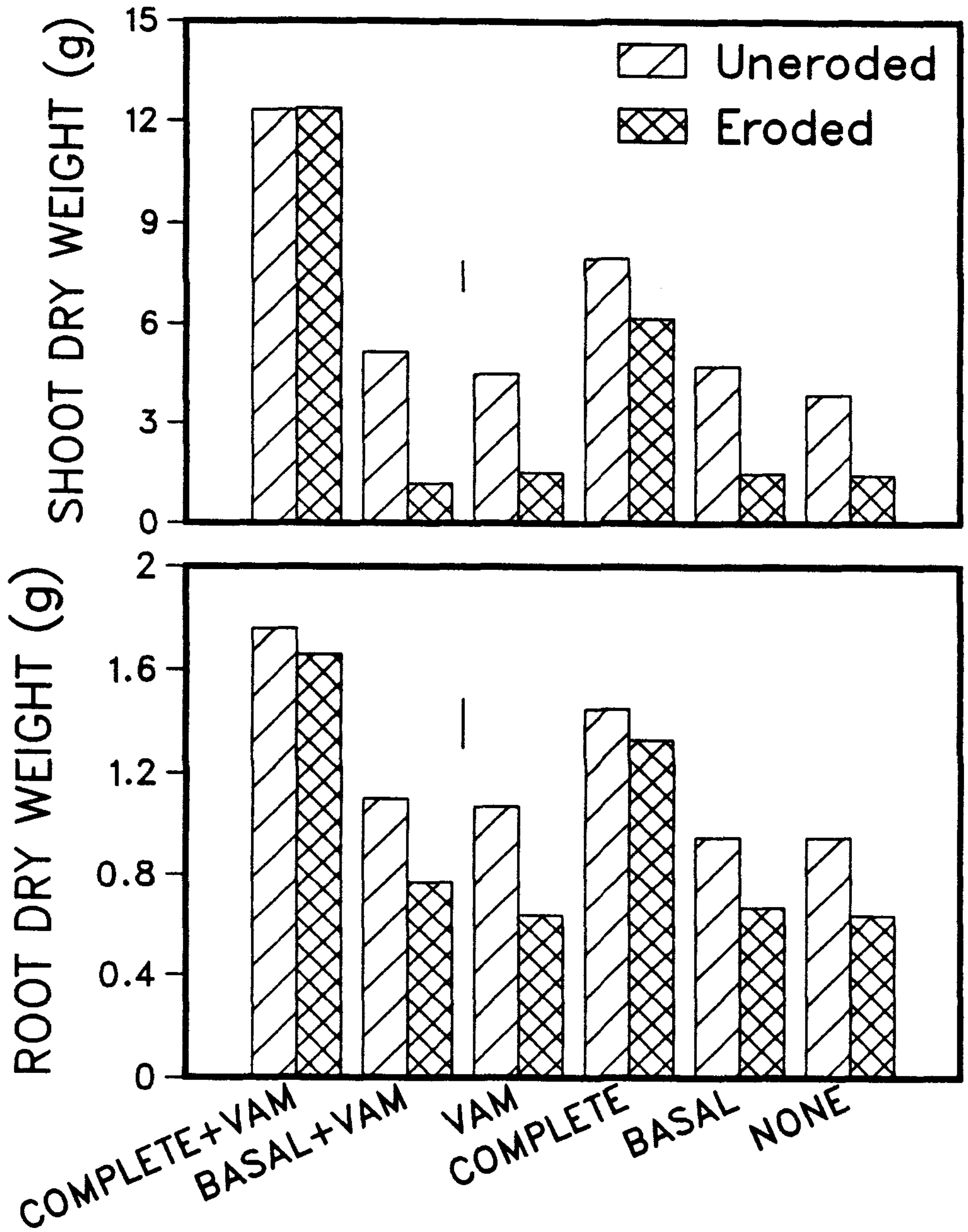

FIG. 8.9. The influence of nutrient amendments and VAM inoculation on dry matter production of cowpea grown in uneroded or eroded soil. Vertical bars represent LSD at the 5% level.

in the eroded soil was lower than in the uneroded soil except when the soil samples were inoculated with G. aggregatum and amended with all the nutrients. Root dry matter production followed similar trends as shoot dry matter production (Fig. 8.9).

The root to shoot ratio of cowpea grown in the eroded soil, whether inoculated or not, decreased signifiantly when the soil was amended with all the nutrients (Fig. 8.10). The root/shoot ratio was lowest when the plants were grown in the presence of G. aggregatum and all the nutrients. There was, however, no change in the root/shoot ratio when plants were grown in the uneroded soil.

Leucaena. Colonization of leucaena roots in the uninoculated soil samples did not change significantly in response to nutrient amendments (Fig. 8.11). Inoculation of the soil samples with G. aggregatum significantly increased the extent of colonization of roots in all the nutrient categories compared to when the soil samples were not inoculated. Amendment of the inoculated soil samples with all the nutrients significantly increased the extent of colonization of roots compared to when the soil samples were amended with only basal nutrients or not amended with nutrients. The extent of colonization of roots was significantly lower in the eroded soil than in the uneroded soil except when the soil samples were amended with all the nutrients and inoculated with G. aggregatum.

Mycorrhizal activity monitored by determining the P content of subleaflets of leucaena grown in the eroded and uneroded soils amended with different nutrient categories is illustrated in Figures 8.12 and 8.13. Mycorrhizal activity in the eroded uninoculated soil did not

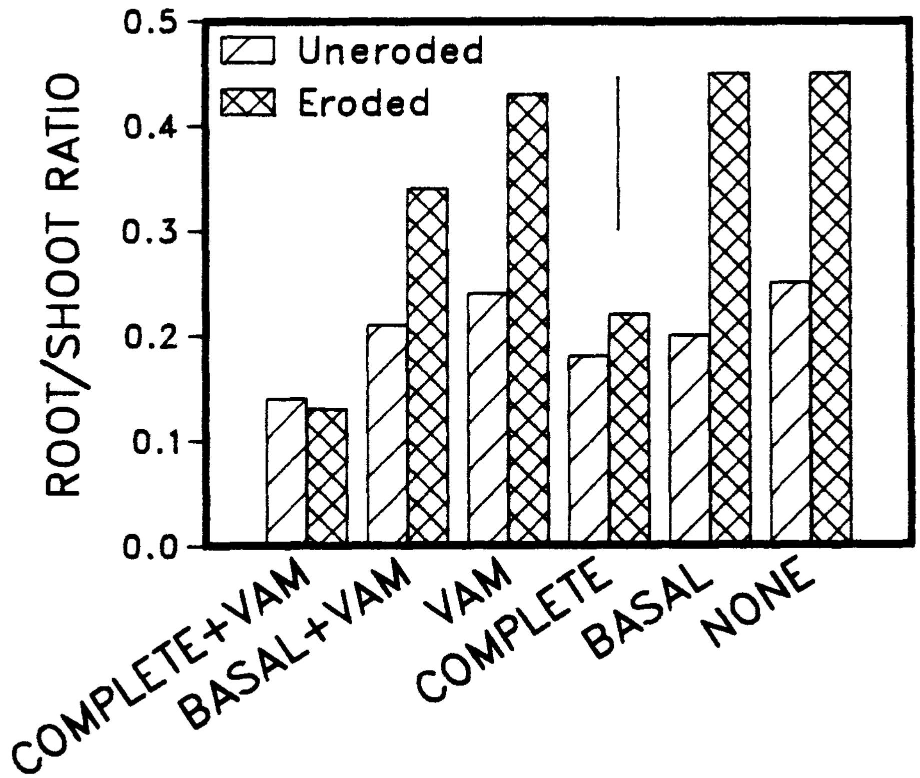

FIG. 8.10. The influence of nutrient amendments and VAM inoculation on root/shoot ratio of cowpea grown in uneroded or eroded soil. Vertical bar represents LSD at the 5% level.

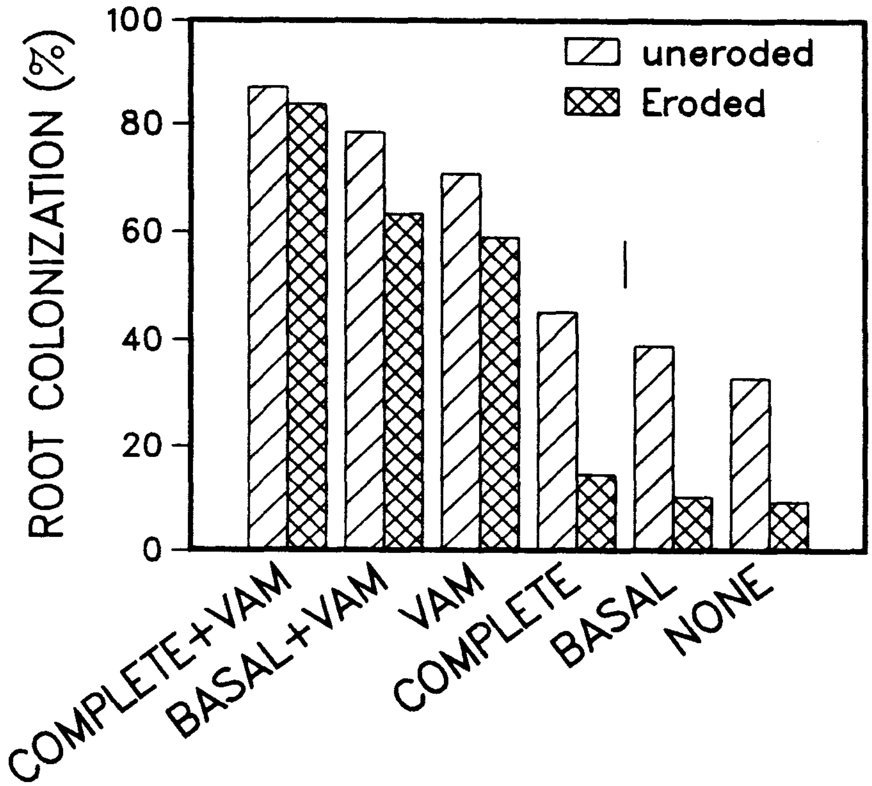

FIG. 8.11. The influence of nutrient amendments and VAM inoculation on the extent of colonization of roots of leucaena grown in uneroded or eroded soil. Vertical bar represents LSD at the 5% level.

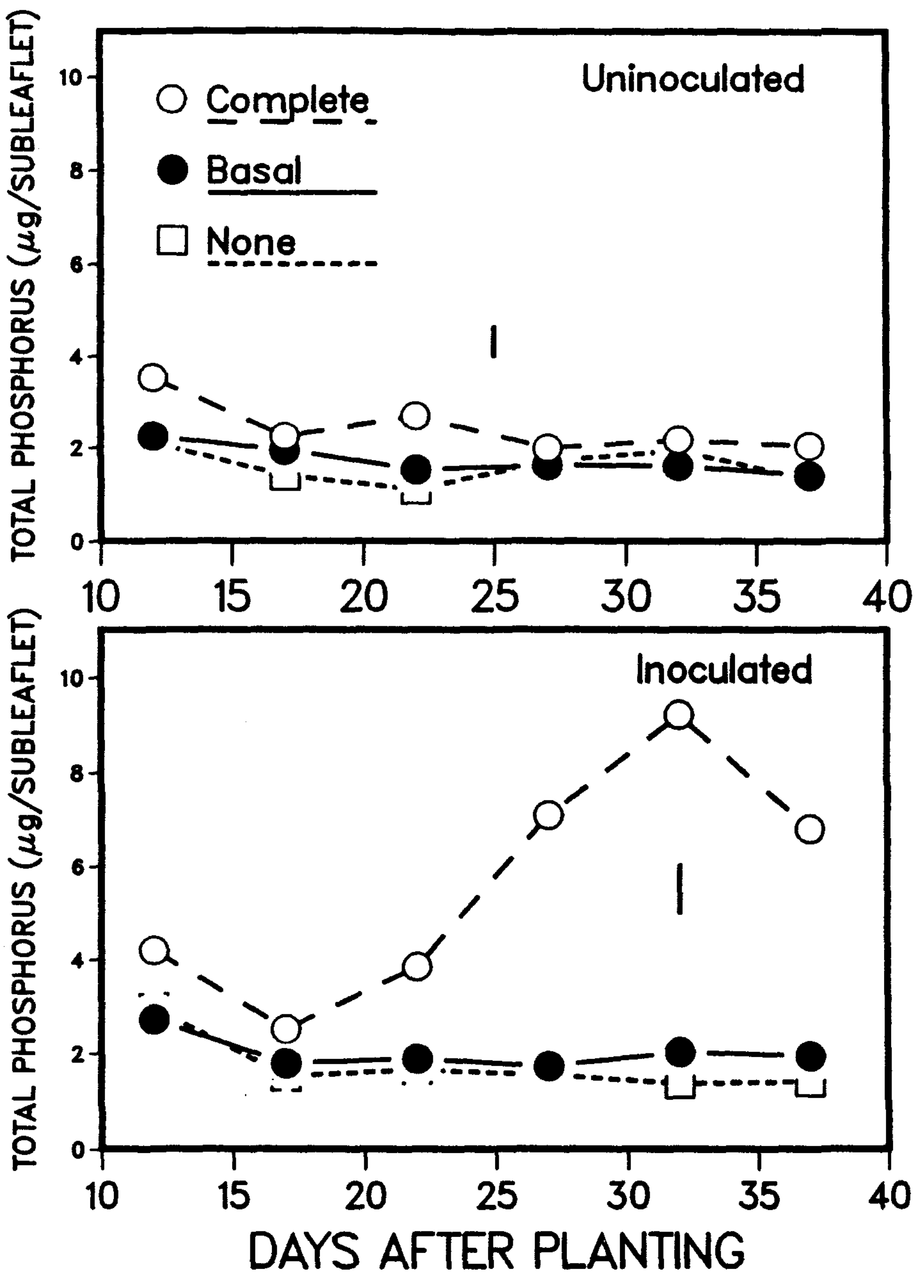

FIG. 8.12. The influence of nutrient amendments and VAM inoculation on the development of mycorrhizal effectiveness in leucaena grown in eroded soil. Vertical bars represent LSD at the 5% level.

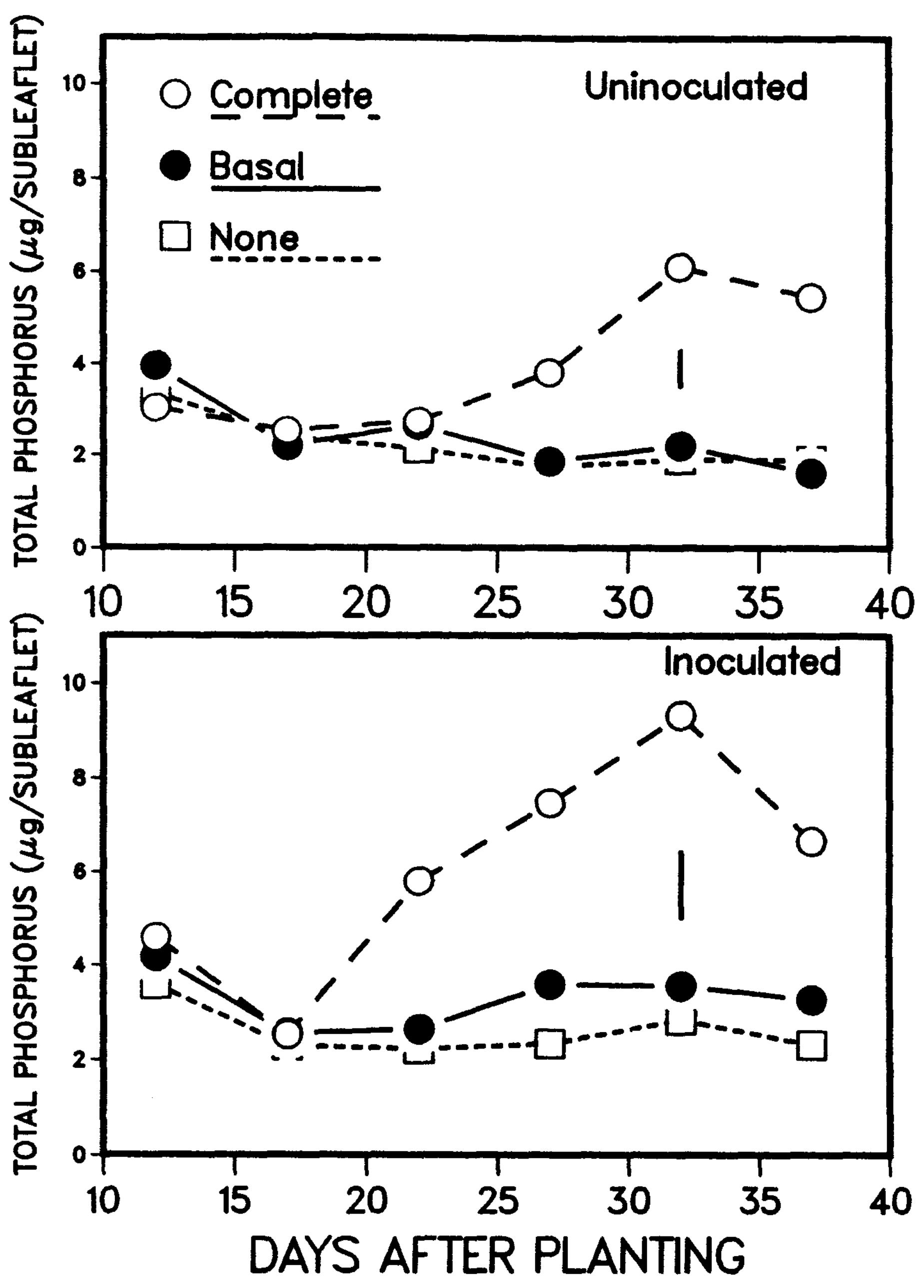

FIG. 8.13. The influence of nutrient amendments and VAM inoculation on the development of mycorrhizal effectiveness in leucaena grown in uneroded soil. Vertical bars represent LSD at the 5% level.

increase when amended with basal nutrients. When the soil was amended with all the nutrients (complete), activity inreased slightly beginning at Day 17 and peaked at Day 22 (Fig. 8.12). The activity observed at this time was significantly greater than that observed in the soil not amended with nutrients. When the soil was inoculated with G. aggregatum in the presence of all the nutrients, the activity was increased by about 3.5 times compared to when the soil was not inoculated. Inoculation of the eroded soil did not affect mycorrhizal activity when the soil was amended with only basal nutrients. Mycorrhizal activity in the uneroded uninoculated soil amended with only basal nutrients was similar to the one observed when nutrients were not added (Fig. 8.13). On the other hand, when the soil was amended with all the nutrients, mycorrhizal activity was detected at Day 22 and peaked 10 days later. At this time, the activity was about 3 times higher than that observed in the soil not amended with nutrients. When the soil was inoculated with G. aggregatum in the presence of all the nutrients, the activity increased by about 50 percent compared to when the soil was not inoculated. Inoculation of the uneroded soil amended with basal nutrients did not lead to significant change in mycorrhizal activity. Similar trends were observed when mycorrhizal activity was monitored in terms of the P concentration of subleaflets [Figures C.7 and C.8 (Appendix C)].

Shoot P concentration of leucaena grown in the eroded uninoculated soil did not increase in response to nutrient amendments (Fig. 8.14). In the uneroded uninoculated soil, however, the shoot P concentration increased in response to the application of all the

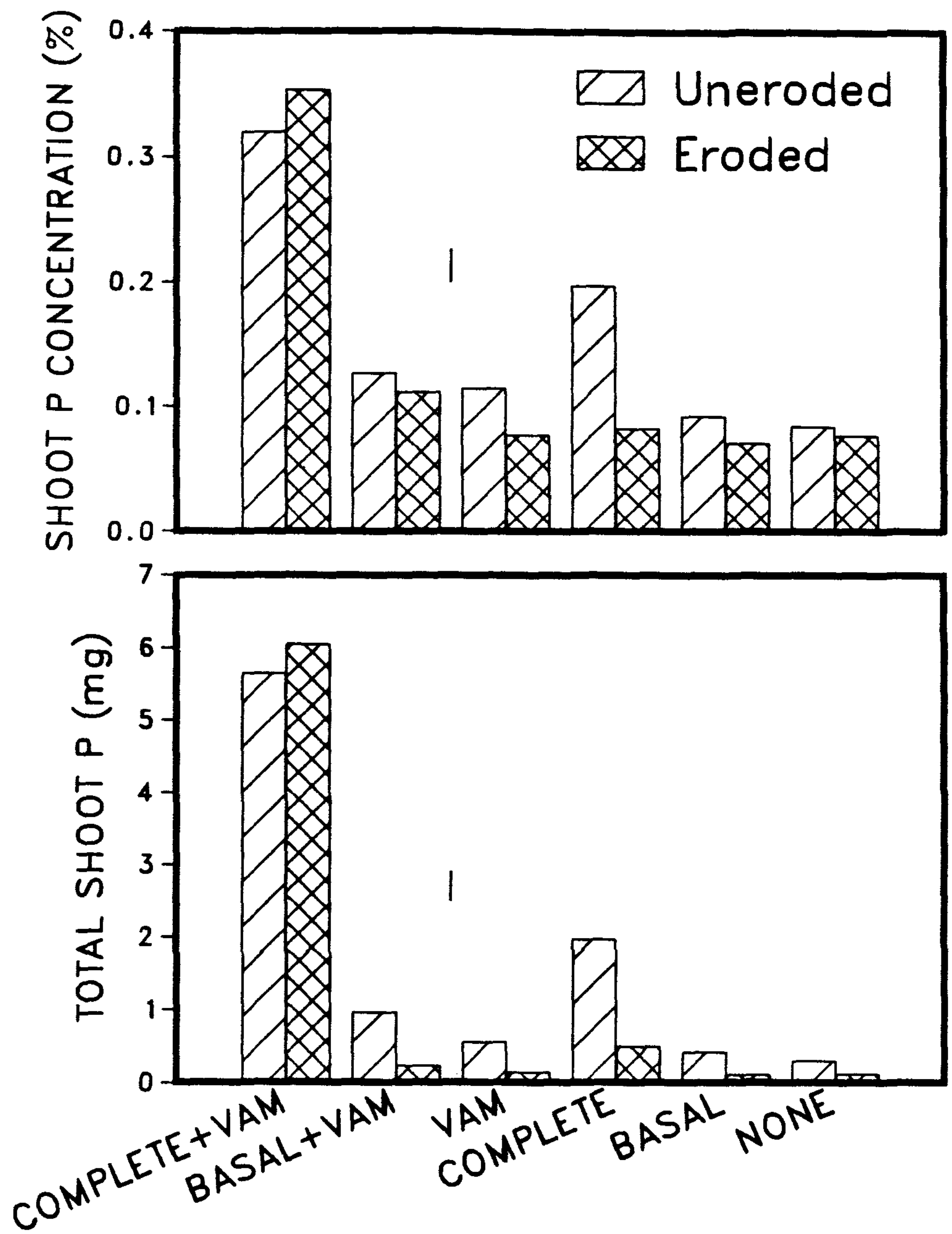

FIG. 8.14. The influence of nutrient amendments and VAM inoculation on shoot P status of leucaena grown in uneroded or eroded soil. Vertical bars represent LSD at the 5% level.

nutrients (complete). When the soil samples were inoculated with *G. aggregatum*, shoot P concentration increased in both the soils when amended with all the nutrients. The highest shoot P concentration was observed when the soil samples were amended with all the nutrients and inoculated with *G. aggregatum*. The trends exhibited by total shoot P content data were similar to that of shoot P concentration.

Shoot Cu concentration of leucaena increased significantly only when the soil samples were inoculated with *G. aggregatum* and amended with all the nutrients (Fig. 8.15). The total shoot Cu content of leucaena, on the other hand, increased when the soil samples were amended with all the nutrients in the presence or absence of *G. aggregatum*. The highest shoot Cu content was observed when the soil samples were amended with all the nutrients and inoculated with *G. aggregatum*.

Shoot Zn concentration of leucaena increased significantly when the soil samples were amended with all the nutrients (complete) in the presence or absence of *G. aggregatum* (Fig. 8.16). The highest level of shoot Zn was observed when the plants were grown in the inoculated soils amended with all the nutrients. The Zn status of plants grown in the eroded and uneroded soils did not differ significantly with any of the treatments imposed. Shoot Zn content of leucaena grown in the uninoculated soil samples increased significantly only in the presence of all the nutrients. When the soil samples were inoculated with *G. aggregatum*, the shoot Zn content of leucaena increased significantly only in the presence of all the nutrients in the eroded soil and in the presence of basal or all the nutrients in the uneroded

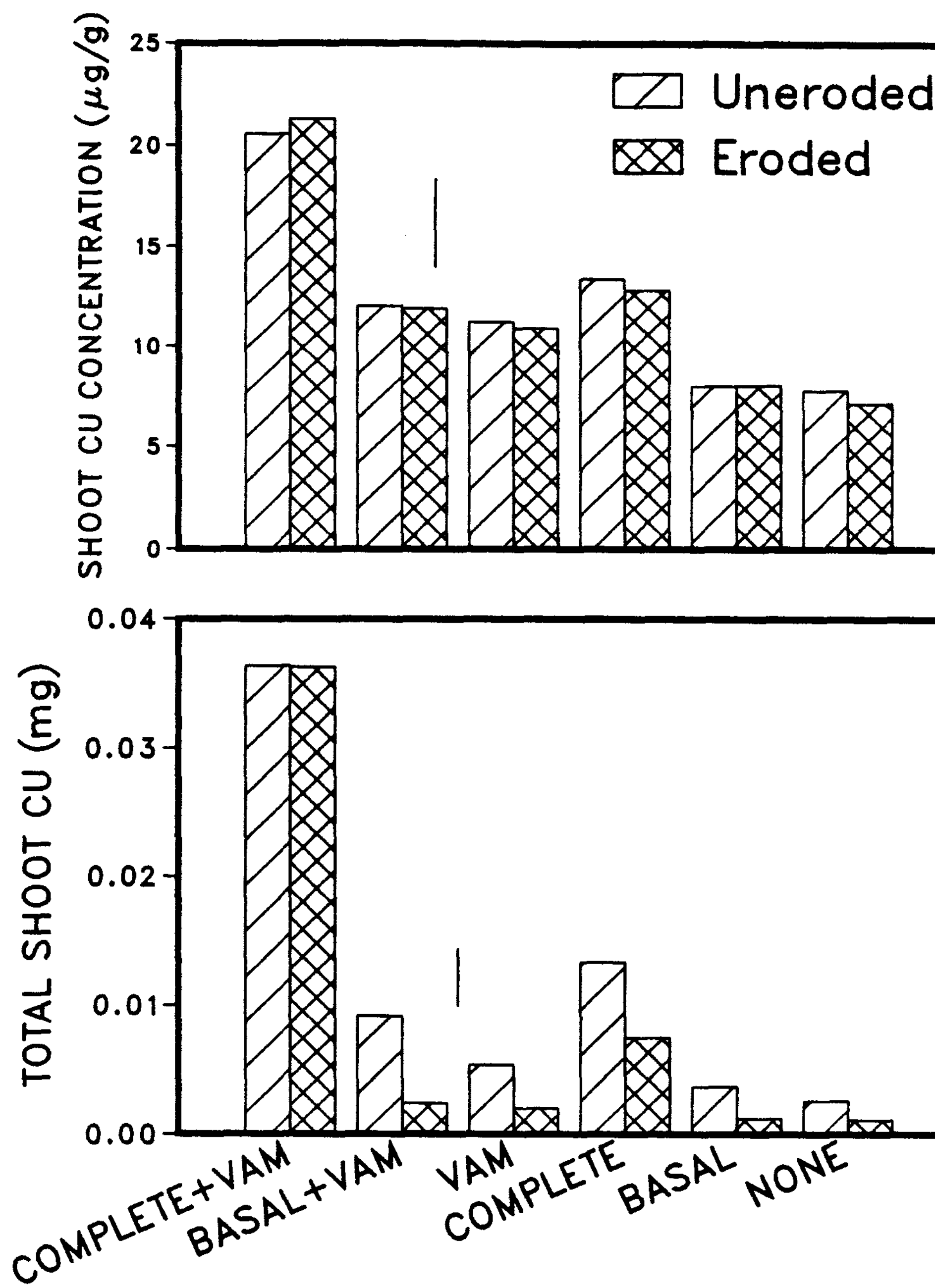

FIG. 8.15. The influence of nutrient amendments and VAM inoculation on shoot Cu status of leucaena grown in uneroded or eroded soil. Vertical bars represent LSD at the 5% level.

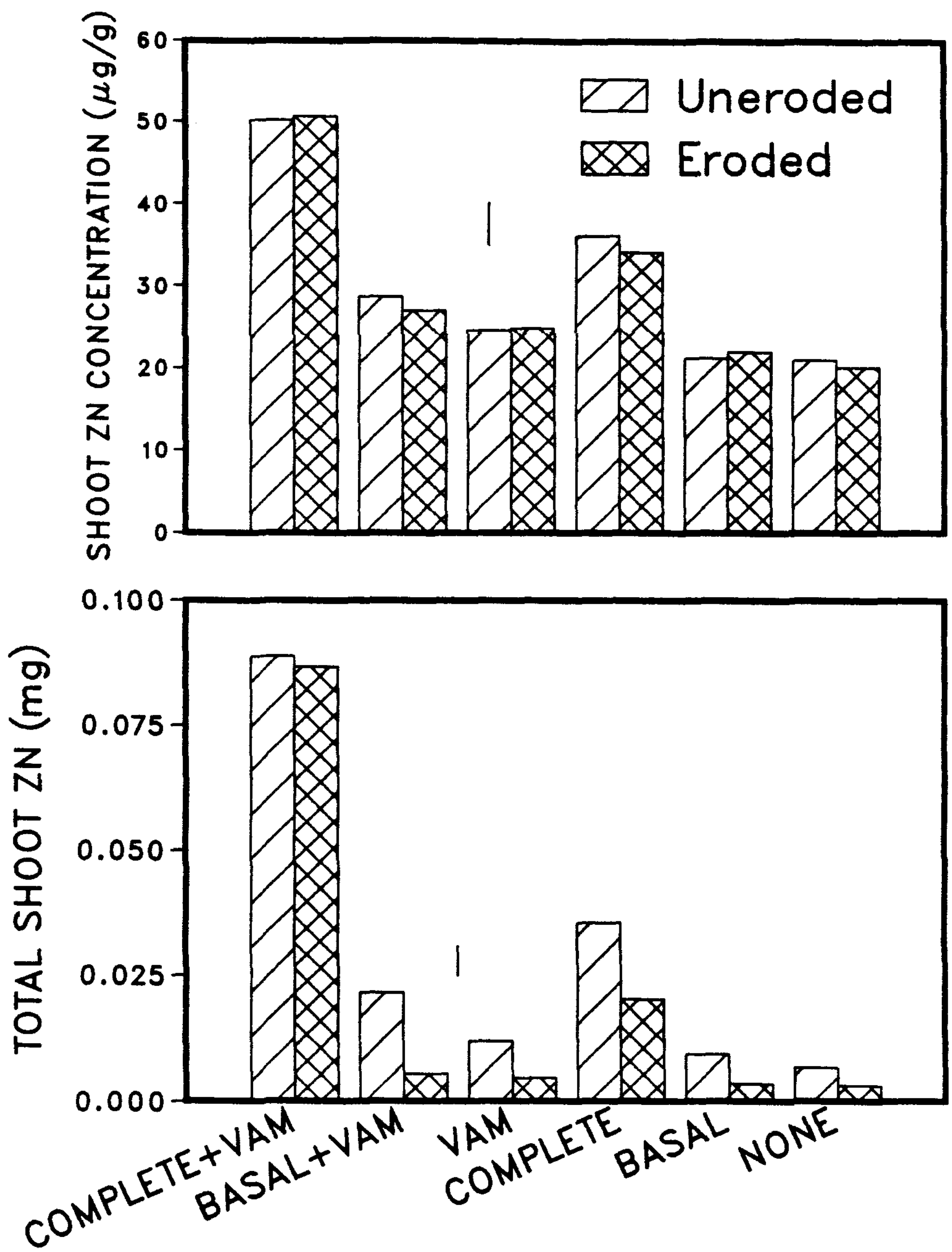

FIG. 8.16. The influence of nutrient amendments and VAM inoculation on shoot Zn status of leucaena grown in uneroded or eroded soil. Vertical bars represent LSD at the 5% level.

soil. The highest level of shoot Zn content was observed when leucaena was grown in the inoculated soil samples amended with all the nutrients.

Nodule dry matter production of leucaena increased significantly only when the soil samples were inoculated with _G. aggregatum_ and amended with all the nutrients (Fig. 8.17). Shoot N concentration of leucaena increased significantly when grown in soils amended with basal nutrients and inoculated with _G. aggregatum_. When the soil samples were amended with all the nutrients, there was a significant increase in shoot N concentration irrespective of inoculation treatment (Fig. 8.18). The maximum shoot N concentration was observed when the legume was grown in the inoculated soil samples amended with all the nutrients. The total shoot N content of leucaena in the uninculated soil samples increased significantly only when amended with all the nutrients. Inoculation of the soil samples with _G. aggregatum_ resulted in an increase in shoot N content of leucaena in the presence of all the nutrients in the eroded soil and in the presence of basal nutrients or all the nutrients in the uneroded soil. The highest shoot N content was observed when the soil samples were amended with all the nutrients (complete) and inoculated with _G. aggregatum_. Shoot N content was lower in the eroded soil than in the uneroded soil except when the soil samples were amended with all the nutrients and inoculated with _G. aggregatum_.

Figure 8.19 illustrates the influence of nutrient amendments on dry matter production of leucaena. Shoot dry weight of leucaena in the uninoculated soil samples increased significantly when the soil

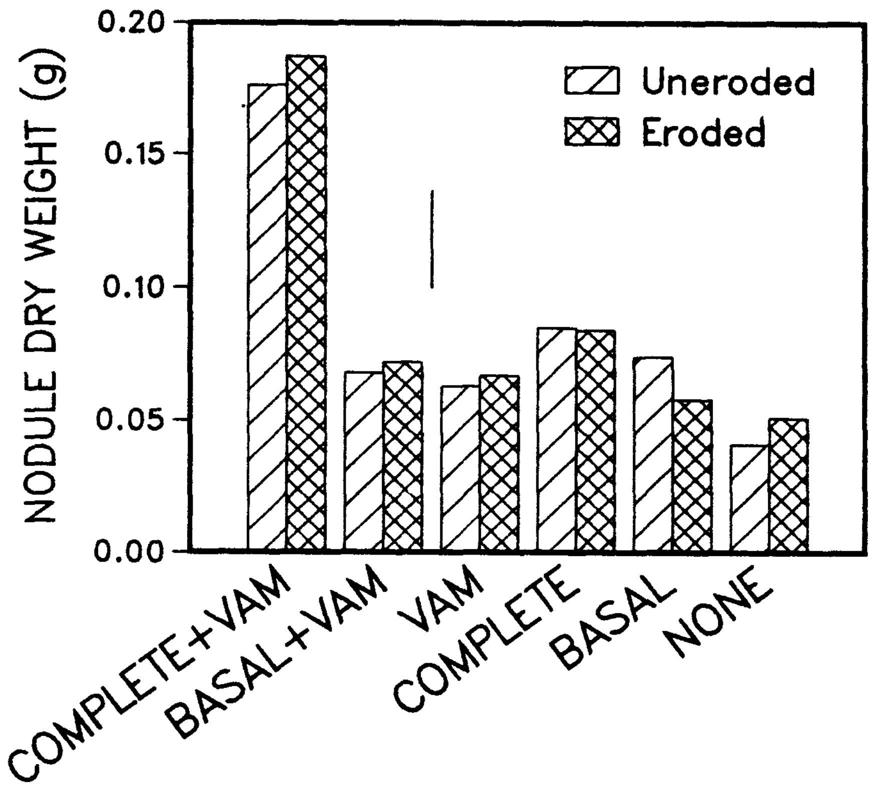

FIG. 8.17. The influence of nutrient amendments and VAM inoculation on nodule dry matter production of leucaena grown in uneroded or eroded soil. Vertical bar represents LSD at the 5% level.

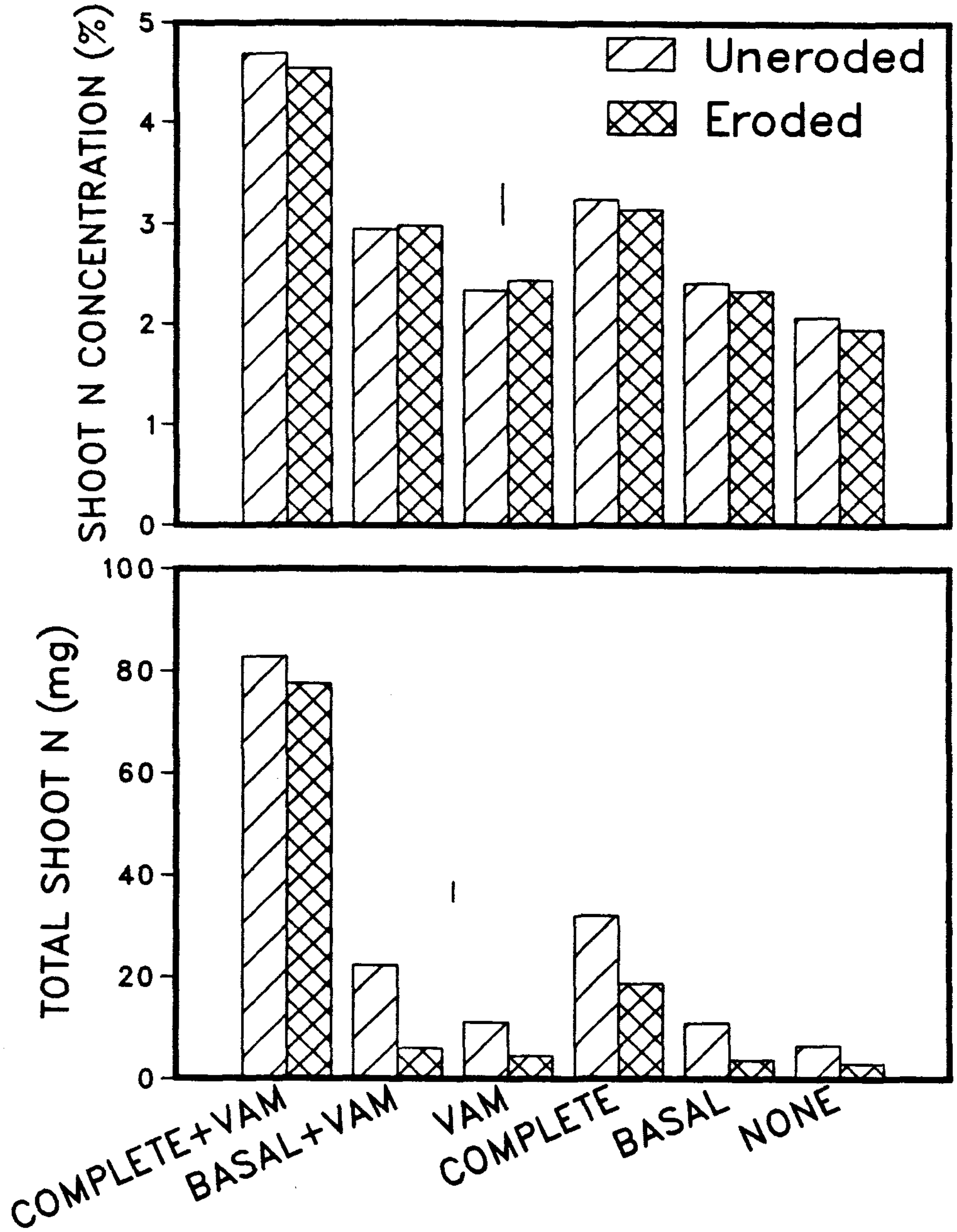

FIG. 8.18. The influence of nutrient amendments and VAM inoculation on shoot N status of leucaena grown in uneroded or eroded soil. Vertical bars represent LSD at the 5% level.

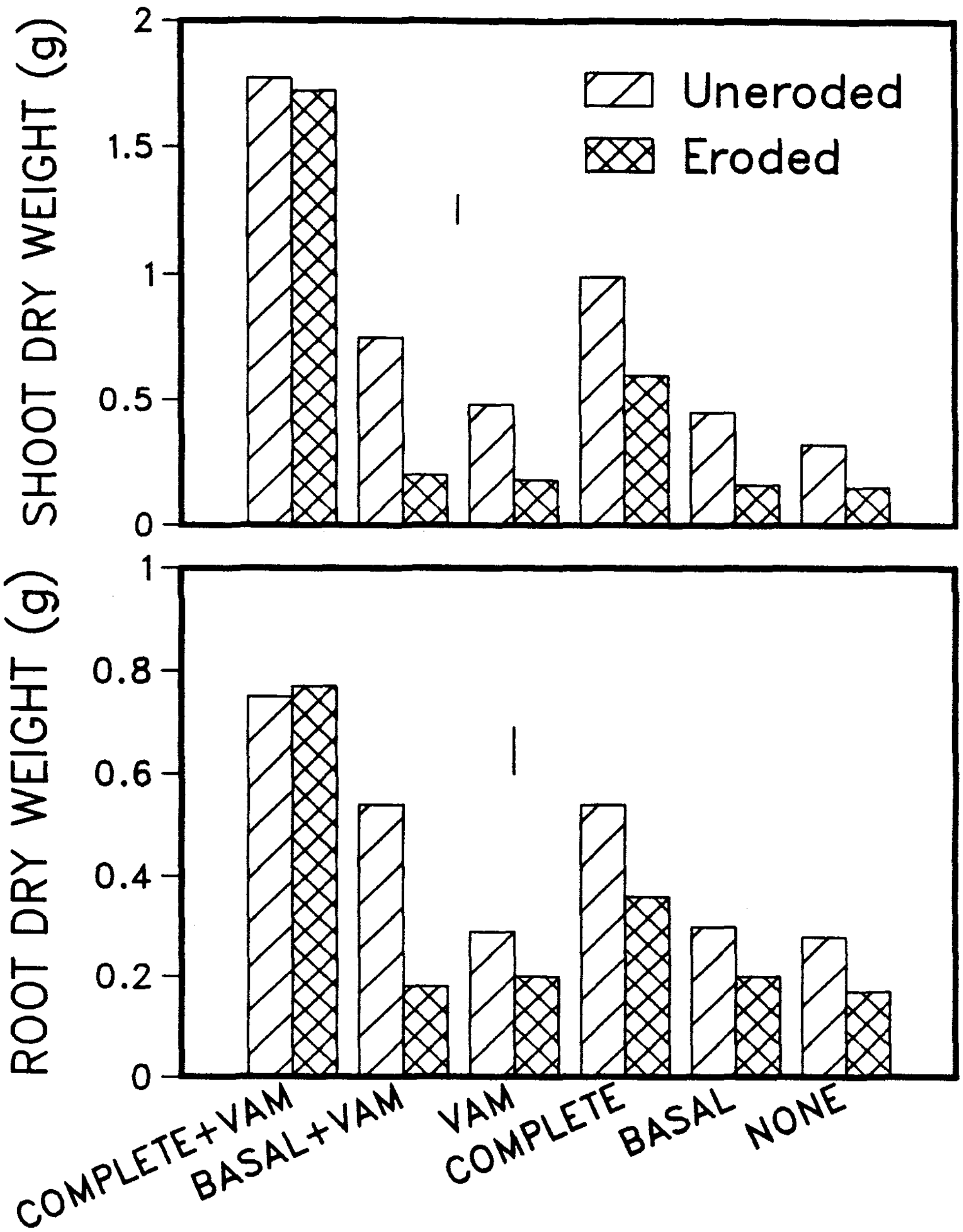

FIG. 8.19. The influence of nutrient amendments and VAM inoculation on dry matter production of leucaena grown in uneroded or eroded soil. Vertical bars represent LSD at the 5% level.

samples were amended with all the nutrients (complete). When the soil samples were inoculated with G. aggregatum, there was a further increase in shoot dry weight in the uneroded soil amended with basal nutrients and in both the soil samples amended with all the nutrients. The highest shoot dry weight was observed when the soil samples were amended with all the nutrients and inoculated with G. aggregatum. Shoot dry weight was lower in the eroded soil than in the uneroded soil except when the soil samples were amended with all the nutrients and inoculated with G. aggregatum. Similar results were obtained for root dry matter production. The root to shoot ratio of leucaena was, in general, lower when the soil samples were amended with all the nutrients (Fig. 8.20).

DISCUSSION

In Chapter 5 it was observed [as has been observed by other workers (20,22,26)] that colonization of roots by VAM fungi increased with increasing levels of soil solution P and decreased at the higher level. The extent of colonization of roots observed in this study was similar to that observed in Chapter 5 when P was applied in optimum amount. Nitrogen also is believed to have an affect on the extent of VAM colonization of roots. Some studies have indicated that root colonization is decreased as a result of N application (10,17,25) while others have shown an increase (9,19). However, Hepper (19) hypothesized that the ratio of N to P is important in determining

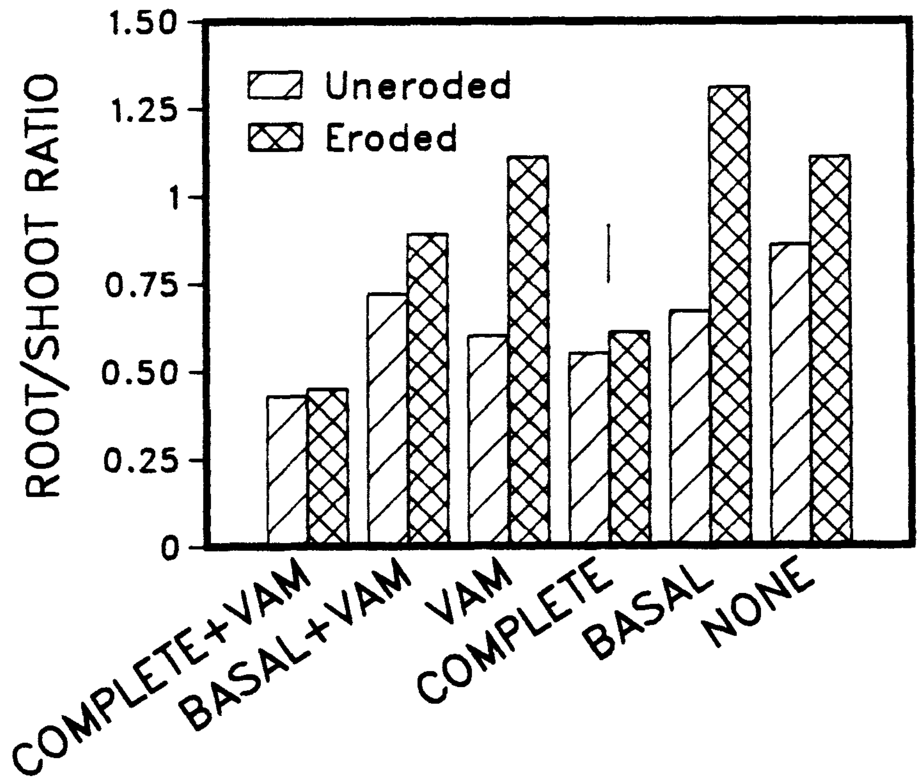

FIG. 8.20. The influence of nutrient amendments and VAM inoculation on root/shoot ratio of leucaena grown in uneroded or eroded soil. Vertical bar represents LSD at the 5% level.

mycorrhizal infection. In the present study, since the extent of VAM colonization of root was above 80%, the P and N nutrition of plants must have been adequate for maximum mycorrhizal infectivity. The fact that the extent of colonization of roots was lower in the unamended eroded soil than in the uneroded counterpart which became similar on addition of nutrients emphasizes the importance of nutrients in mycorrhizal colonization.

Since mycorrhizal activity associated with the application of basal nutrients was small in the uneroded soil and absent in the eroded soil, the increase in VAM activity observed as a result of application of all the nutrients must be due to the effects of P, N and lime. It was observed in Chapters 5, 6 and 7 that amendment of the eroded soil with P at the optimum level increased mycorrhizal activity of cowpea and leucaena by 6 and 10 folds, respectively, while liming had no effect. Application of inorganic N had no effect on cowpea but the activity was increased by 2 folds in leucaena. Differences in mycorrhizal activity observed between cowpea and leucaena in response to inorganic N is, probably, due to differences in host species. It can, thus, be deduced from the above mentioned facts that most of the activity observed was due to the addition of P. Similar conclusion could be reached if shoot dry matter data is considered. Role of soil solution P in determining mycorrhizal activity in cowpea and leucaena is well established (4,16). Soil solution P levels of 0.026 and 0.021 mg/l have been reported to be optimum for cowpea and leucaena, respectively (4,16). Comparable mycorrhizal activity was observed in the present study when P was applied to obtain levels similar to the above mentined values for complete nutrients.

It has been clearly demonstrated in this study that mycorrhizal activity is dependent on the nutrient content of soil. Plants grown in the eroded soil, which was low in most nutrients, were not responsive to VAM inoculation unless the soil was amended with nutrients. In the uneroded soil, on the other hand, which had a better nutrient status to start with, plants were responsive. These results are indicative of two facts. Firstly, there seems to exist minimum levels of nutrients such as P and N that are necessary for mycorrhizal activity; secondly, mycorrhizal activity in eroded soils could be improved or restored to the level of the uneroded soil by compensating them for losses of nutrients accompanying erosional soil losses in addition to compensating them for losses of VAM fungi. The low mycorrhizal activity observed in the unamended soil samples (particularly in the eroded soil) or the soil samples amended with only basal nutrients could be explained from the presence of threshold levels of P and N. Suppression in shoot dry matter production of cowpea and leucaena in the eroded unamended soil could similarly be explained. It can be seen from the results that when the soil samples were amended with nutrients determined to be optimum for symbiotic effectiveness, almost half of the shoot dry matter production was associated with VAM inoculation. The other half appears to be a result of the nutrients added and the inherent growth potential of the soil. Thus, the presence of nutrients as well as VAM fungi appears to be necessary for establishing legumes in eroded Wahiawa soil and attain growth comparable to that observed in the uneroded soil. Habte et al. (15) have recently emphasized the significance of nutrient amendments of eroded soils along with VAM inoculation as a necessary step towards rehabilitating eroded soils. Importance of VAM inoculation and nutrient amendment to soil can also be visualized from root/shoot ratios. Plants grown in the eroded soil in the absence of nutrients or introduced endophytes have a high root/shoot ratio indicating that the plants are under stress and the growth of roots has been stimulated to absorb more nutrients. The low root/shoot ratio values are typical of the mycorrhizal plants as observed in this study (3,26).

The increase in mycorrhizal activity observed at about 30 DAP when leucaena was grown in the uninoculated uneroded soil amended with all nutrients is due to the presence of native soil VAM fungi. Activity was eventually increased further upon inoculation of soil with G. aggregatum which indicates that leucaena was responsive to inoculation of soil with G. aggregatum in the presence of native VAM population.

Increase in the uptake of immobile nutrients such as P, Cu and Zn and subsequent increase in plant growth due to VAM inoculation has been observed by others (1,5,7,11,12,14,21,23,24). An overall similarity in shoot status of Zn and Cu to that of shoot P status observed in this study underline the importance of VAM fungi in the uptake of immobile nutrients other than P. When soil samples (particularly the eroded soil) were not amended with nutrients or inoculated with VAM fungi, the extent to which the shoot uptake of nutrients was reduced was much greater than the reduction observed in shoot concentration. This is, probably, a result of concentration effect, i.e., plants growing in the absence of complete nutrients or VAM fungi have a higher concentration of nutrients because of their small dry matter production. So, both nutrient concentration and nutrient content of shoots should be taken into account when considering mycorrhizal effectivity.

The present results indicate that adequate nutrition (especially inorganic N and P) and VAM inoculation are necessary for improved nodulation. Application of starter N promotes nodulation by overcoming N deficiency during the establishment of N_2-fixation process (13). This would particularly be true in eroded soils because of their low N content. Increase in nodulation due to addition of P to soil along with VAM inoculation is well documented (2,27). Since P was added as part of the complete nutrients in the present study, it is one of the nutrients that has led to increased nodule dry weight. Inoculation of soils with VAM fungi, especially in P deficient conditions, ensures the availability of extra P needed for N_2-fixation. This is further confirmed from the fact that shoot P status of cowpea or leucaena was highest when grown in soils amended with all the nutrients and inoculated with VAM fungi. Similarity in trends of nodule dry weight and shoot N concentration indicate that the nodules were active in N_2 fixation.

The results of this study demonstrate the benefits of VAM inoculation in growing nodulated legumes in eroded soils. Phosphorus is, perhaps, the most important factor associated with enhanced VAM activity and/or host growth. This finding has been confirmed using two legumes i.e. cowpea and leucaena. Hence, in order to derive maximum benefits for growing effectively nodulated legumes in eroded soils, amendment of soil with nutrients in optimum amounts and inoculation with VAM fungi are very important.

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SUMMARY

Because of the adverse effect of erosion on soil productivity, scientists have sought effective ways to rehabilitate eroded soils. Most of the efforts have been through chemical methods, i.e., by adding fertilizers. This is an expensive method, especially, for farmers of the tropics. On the other hand, rehabilitating eroded soils by biological methods, (i.e. by inoculating soils with VAM fungi and rhizobia with minimum chemical inputs) has received little attention. The latter method is more likely to be feasible in the tropics. The objective of this study, therefore, was to determine the role of VAM fungi in reducing chemical inputs and to define the levels of those chemical inputs necessary for successful establishment of effectively nodulated and mycorrhizal cowpea and leucaena in an eroded tropical soil.

Erosion was simulated by removing the top 30 cm of the Wahiawa soil (Tropeptic Eutrustox). Removal of top soil resulted a 78 and 86 percent decrease in VAM spores and infective propagule numbers per g of soil, respectively. The extent of VAM infection in roots of native vegetation was also lower in the eroded soil. These results indicate that the VAM inoculum potential is reduced significantly as a result of simulated erosion.

The soils were then inoculated with 3 species of VAM fungi (<u>G. aggregatum</u>, <u>G. mosseae</u> or <u>G. etunicatum</u>) with no chemical amendment to determine their potential for improving yields of cowpea and leucaena

under eroded conditions. The results showed that inoculation of the uneroded soil with G. aggregatum increased mycorrhizal activity, nodule dry weight and plant growth compared to that of the uninoculated control. None of the VAM fungi tested significantly influenced plant response in the eroded soil. These results and the fact that nutritional status of eroded soils are poor indicated that low nutrient content was the limiting factor in the eroded soil.

In the next series of experiments, soils were amended with nutrients to determine the effects of chemical inputs and VAM inoculation on crop productivity on an eroded soil. The nutrients/chemicals amended were P, inorganic N, lime, Mo, and organic residue.

Application of P to bring soil solution P up to 0.026 mg/l increased the extent of VAM colonization of roots. As the level of soil solution P increased above 0.046 mg/l, the extent of colonization of roots decreased. Mycorrhizal activity (determined on the basis of P status of leaf discs or subleaflets) was observed only in the uneroded soil in the absence of added P but when the soil solution P level was raised to 0.026 mg/l, mycorrhizal activity was also observed in the eroded soil. This observation indicated that a threshold level of P was required for mycorrhizal activity. Maximum mycorrhizal activity was observed at the soil solution P level of 0.026 mg/l. Shoot P content and dry matter yields in the eroded soil increased significantly with the addition of P and became similar to that of the uneroded soil at a soil solution P level of 0.026 mg/l. Maximum nodule dry weight was observed when the soil solution P level was increased to 0.046 mg/l.

Liming the eroded soil to pH 6.0 increased the extent of VAM colonization of roots significantly. Above pH 6.0 there was no significant change. In the uneroded soil, on the other hand, liming did not increase the extent of VAM colonization of roots. Mycorrhizal activity also was not influenced by liming. The pattern of shoot dry weight was similar to that of root colonization. The results indicated that liming was beneficial only in the eroded soil upto pH 6.0.

Amendment of the eroded soil with organic residue did not influence the growth of cowpea but the growth of leucaena was reduced when soil samples were amended with high levels of organic residue (7.38%). Reduction in plant growth was associated with increase in shoot Mn content. The results indicated that amendment of the eroded Wahiawa soil with organic residue is not beneficial to the growth of mycorrhizal cowpea or leucaena. Application of Mo to soil also did not influence mycorrhizal activity or plant growth. It appears from the results that application of Mo is not necessary for the growth of mycorrhizal cowpea or leucaena in the eroded Wahiawa soil when amended with other nutrients.

Application of inorganic N increased the extent of VAM colonization of cowpea and leucaena roots. Optimum N level for mycorrhizal activity was found to be 25 ppm. Nodule dry matter production and shoot N content increased with increasing levels of inorganic N reaching a maximum value at 50 ppm. Dry matter yields were low in the eroded soil in the absence of added N. BY adding N, the dry matter yields increased significantly and both the soils had similar shoot and root dry matter yields. The results indicated the potential beneficial effect of starter N application on the growth of mycorrhizal cowpea and leucaena in eroded soils.

In the last experiment, levels of nutrients determined to be optimum for mycorrhizal activity in previous experiments were combined and tested for mycorrhizal activity and plant growth. The treatments consisted of growing cowpea or leucaena in soils amended with either complete or basal or no nutrients in the presence or absence of G. aggregatum. The extent of VAM colonization of roots increased due to inoculation of soils with G. aggregatum. The highest level of colonization was observed in soil amended with all the nutrients and inoculated with VAM fungi. Application of basal nutrients alone to soil did not improve mycorrhizal activity. Mycorrhizal activity in the uneroded uninoculated soil increased when amended with all the nutrients and increased further on inoculation with G. aggregatum. In the eroded soil, mycorrhizal activity increased only when amended with all the nutrients and inoculated with G. aggregatum. These results illustrate that maximum mycorrhizal activity in the eroded soil can only be achieved if it is amended with all the nutrients and inoculated with VAM fungi. Patterns of shoot P, Cu and Zn status were, in general, similar to those (the patterns) of mycorrhizal activity. Nodule dry matter production, shoot N status and shoot and root dry weights were increased when the soil samples were amended with all the nutrients and inoculated with G. aggregatum.

The results of this study demonstrate the benefits of VAM inoculation in growing nodulated legumes in eroded soils. The results also suggest that the increase in growth due to amendment of soil with all the nutrients was mainly due to the addition of P, N, and lime.

Hence, in order to derive maximum benefits for growing effectively-nodulated legumes in eroded soils, amendment of soil with nutrients (P, N and lime) and inoculation with VAM fungi are essential.

APPENDIX A

EFFECT OF SIMULATED EROSION ON SOME SELECTED SOIL PROPERTIES

MATERIALS AND METHODS

Physical properties. The water holding capacity of soil was determined by placing a moist filter paper cut into right size into a porcelin crucible with perforations at the bottom. The crucible was weighed and filled with oven dried soil and compacted by dropping it 10 times from a distance of approximately 3 cm. The soil surface was labelled off with a spatula. After weighing again, the crucible filled with soil was placed in a chamber containing a layer of water deep enough to dip the bottom of the crucible. After about 2 hours, when the soil samples were saturated with water (water appear on soil surface), the crucible was removed and placed in a humid closed chamber in order to drain off the excess water. The crucible was weighed again. Water holding capacity of soil was calculated by determining the net amount of water held by an unit weight of dry soil. The particle size fraction of soil was determined by the pippett method as described by Gee and Bawder (6).

Chemical properties. For the measurement of soil pH, 15 g portion of the soil sample was transferred into a 50 ml beaker. Thirty ml portions of deionized water were added to it and stirred well with a glass rod. After 30 minutes of standing, the pH was measured using a pH meter (Model Fisher 805 MP, Fisher Scientific Company, Pittsburgh, Pennsylvania 15219) by dipping the glass electrode to the supernatant liquid for 15 seconds.

Organic carbon content of the soil was determined by the "dry combustion method". The soils were passed through a 250 m sieve (#60) and oven dried at 110 for 48 hours. The soil samples were then combusted and organic carbon determined automatically by the Carbon Determinator (WR-112), Leco Corporation, St. Joseph, MI. To determine the organic matter content of soil, the value obtained for organic carbon was multiplied by a factor of 1.9.

Total soil nitrogen was determined by the micro kjeldahl digestion and steam distillation of NH_3 (2). Inorganic nitrogen (ammonium- and nitrate-N) was determined by steam distillation of KCl soil extract (8). Available soil phosphorus was determined after extraction with 0.01M $CaCl_2$ following the procedure described by Fox and Kamprath (4).

Exchangeable calcium, magnesium, potassium and sodium were determined after extracting soil samples with 1N ammonium acetate and reading the concentrations by atomic absorption spectroscopy (9). For the determination of extractable manganese, the method described by Fox et al. (5) was used. In this method, the soil samples were extracted with 1N KCl and the concentration of Mn determined by atomic absorption spectroscopy. Extractable molybdenum was determined by the "acid ammonium oxalate method" (10). Twenty five g portions of soil was shaken with 250 ml of acid ammonium oxalate for 8-10 hours and then filtered through Whatman No. 40 filter paper that had been washed with 6.5 M HCl. First 10 to 15 ml of filtrate was discarded. Molybdenum was determined from the filtrate using the "Induced Plasma Emission Spectroscopy" on a Perkin-Elmer Model 6500 ICP/AES (Inductively coupled Plasma/Atomic Emission Spectroscopy, Perkin-Elmer, Norwalk, Connecticut).

Lime requirement curves were constructed using $Ca(OH)_2$ and following the procedure described by Coleman and Thomas (3). The P-sorption capacity of the soil samples was determined before and after liming after sieving the soil samples through a 1-mm sieve (#16). The procedure followed was that of Fox and Kamprath (4).

Biological Properties. The population of bacteria in soil was enumerated by plate counts on tryptic soy agar with 200 ppm actidione. The plates were incubated at 30 C for 3 days before counts were made. The actinomycete population was determined by plate counts on Jensen's medium. The plates were incubated at 30 C for 8 days and then the colonies were counted. Fungal populations in soil were estimated by plate counts on Rose bengal agar supplemented with 30 ppm streptomycin. The plates were incubated at 30 C for 3 days before counts were made. Protozoan populations in soil were determined as described by Habte and Alexander (7). Protozoa were counted by the most probable number method by recording the numbers of rings with or without protozoa.

For the measurement of native plant roots in soil, the soil samples were collected from the field using auger from 15 different locations within the soil collection site. Soils from 5 locations were mixed together to make 3 replications. The roots were separated from soil, washed with water and collected by a floatation method (1). The roots were dried at 70 C for 48 hours. Root length was determined by the line intersect method (11) and expressed as cm of root per g dry soil. Dry weight of native roots in soil was also determined.

RESULTS

Physical properties. The influence of simulated erosion on soil physical properties examined is shown in Table A.1. The water holding capacity of soil increased by 8.4% due to erosion but the increase was not statistically significant. The particle size fractions of soil changed significantly due to top soil removal. The sand and silt fractions decreased by 71 and 59%, respectively, whereas the clay fraction increased by 44% as a result of simulated erosion.

Chemical Properties. The influence of simulated erosion on pH, organic carbon, total and inorganic nitrogen and available phosphorus contents of soil is shown in Table A.2. The pH decreased significantly from 5.89 to 5.40 due to top soil removal. Simulated erosion reduced the organic carbon and total nitrogen contents of soil by 42 and 52 percent, respectively. In contrast, the NO_3-N content was increased by 82% but the increase was not statistically significant. The NH_4-N content, however, didnot change due to top soil removal. Simulated erosion also resulted a 67% reduction in the

TABLE A.1. Influence of simulated erosion on water holding capacity and particle size fraction of soil[a]

Soil	Water holding capacity (%)	Particle size fraction (%)		
		Sand	Silt	Clay
Uneroded	57.18 a	14	27	59
Eroded	61.96 a	4	11	85

[a]Means followed by the same letter within a column are not significantly different from each other at the 5% level.

TABLE A.2. Influence of simulated erosion on some chemical properties of soil[a]

Soil	pH	Organic carbon (%)	Total N (%)	Inorganic N (ppm)		Available p (mg/l)
				NO_3-N	NH_4-N	
Uneroded	5.89a	1.94a	0.26a	12.8a	18.7a	0.009a
Eroded	5.40b	1.13b	0.13b	23.3a	18.7a	0.003a

[a]Means followed by the same letter within a column are not significantly different from each other at the 5% level.

soil solution P content of soil. The decrease was, however, not statistically significant.

Table A.3 shows the influence of simulated erosion on macro- and micronutrient contents of soil. Simulated erosion resulted a significant decrease in the concentrations of exchangeable Ca, Mg, K and Na, respectively. The concentrations of extractable Mn and Mo were also reduced when the soil was subjected to simulated erosion. The decrease was statistically significant for Mn but not for Mo.

Lime requirement curve of soil did not change much due to removal of top soil (Fig. A.1). Phosphorus sorption curves of soil established at pH 6.0 and 6.5 are shown in Fig. A.2. The curve was higher for the eroded soil than for the uneroded soil.

Biological Properties. Soil losses associated with simulated erosion were not accompanied by significant changes in the numbers of total bacteria while they were associated with significant reduction in the populations of soil actinomycetes, fungi and protozoa (Table A.4). The population of bacteria was reduced by 76% (not significant) whereas the populations of actinomycetes, fungi and protozoa were decreased by about 100- 10- and 10-folds, respectively, due to top soil removal.

Although the native soil root weight and length were reduced by 80 and 90 percent, respectively due to simulated erosion, the results were not significant (Table A.5). When the root length was calculated in terms of the unit root weight, the results obtained were 50% lower in the eroded soil than in the uneroded soil.

TABLE A.3. Influence of simulated erosion on some macro and micronutrient content (in ppm) of soil[a]

Soil	Ca	Mg	K	Na	Mn	Mo
Uneroded	948.2a	211.9a	116.5a	90.6a	39.9a	0.414a
Eroded	504.3b	100.3b	30.7b	72.7b	21.7b	0.230a

[a]Means followed by the same letter within a column are not significantly different at the 5% level.

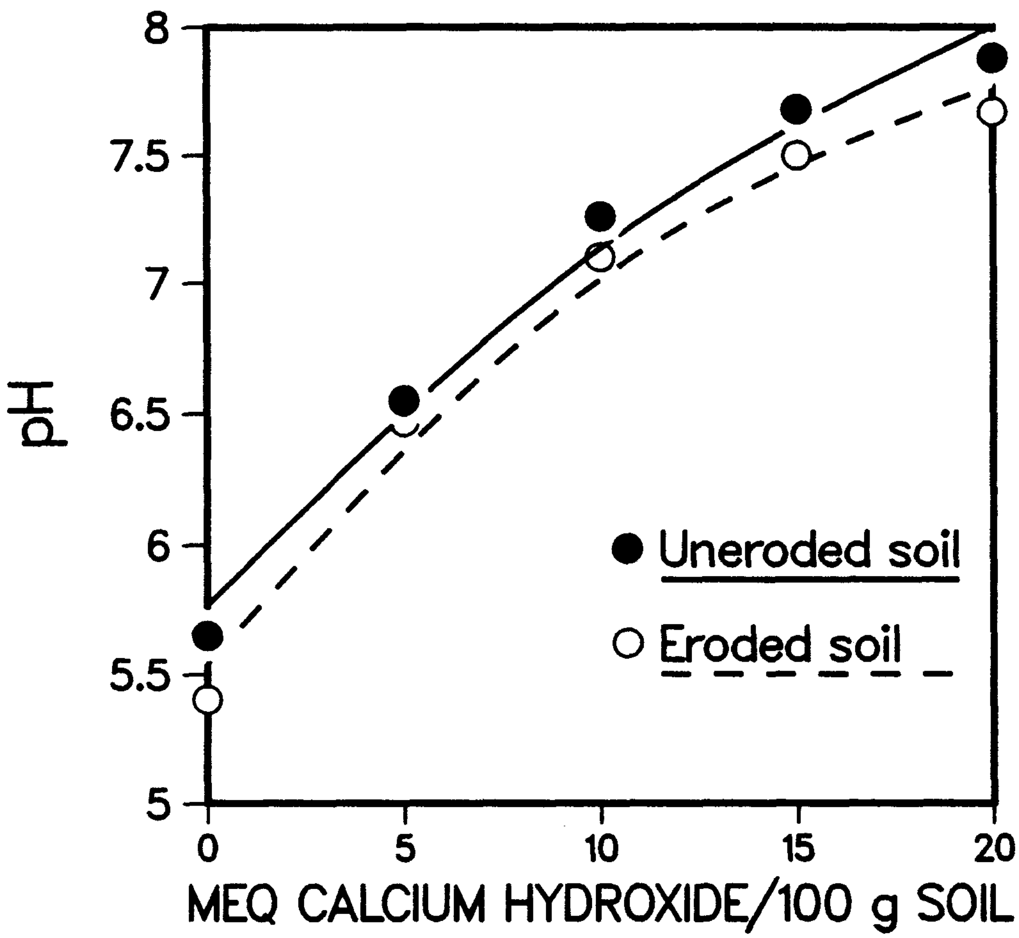

FIG. A.1. The lime requirement curves for eroded and uneroded soil.

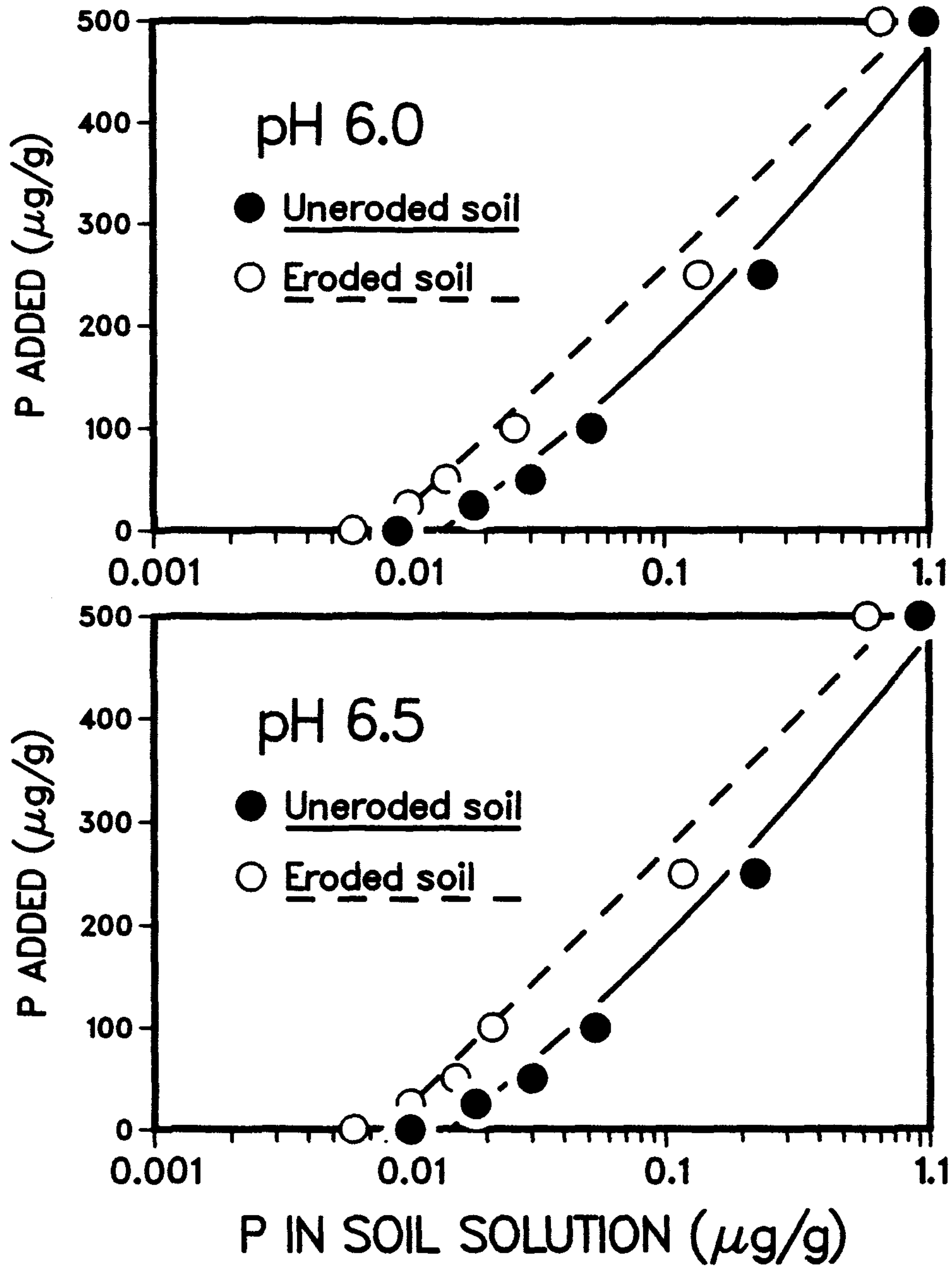

FIG. A.2. The P sorption curves for eroded and uneroded soil.

TABLE A.4. Influence of simulated erosion on the population of some microorganisms in soil[a]

Soil	Bacteria	Actinomycetes	Fungi	Protozoa
	Number of colony forming units per gram of soil			
Uneroded	3.7×10^5a	1.9×10^6a	9.3×10^4a	1.4×10^3a
Eroded	0.9×10^5a	5.1×10^4b	8.5×10^3b	1.5×10^2b

[a]Means followed by the same letter within a column are not significantly different at the 5% level.

TABLE A.5. Influence of simulated erosion on the native root content of soil[a]

Soil	Root weight/g soil	root length/g soil	root length/g root
Uneroded	0.003a (g)	20.28a (cm)	6510.9a (cm)
Eroded	0.0006a (g)	1.96a (cm)	3270.0a (cm)

[a]Means followed by the same letter within a column are not significantly different from each other at the 5% level.

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APPENDIX B

CONCENTRATION OF P IN COWPEA LEAF DISCS (FIG. 1-9)

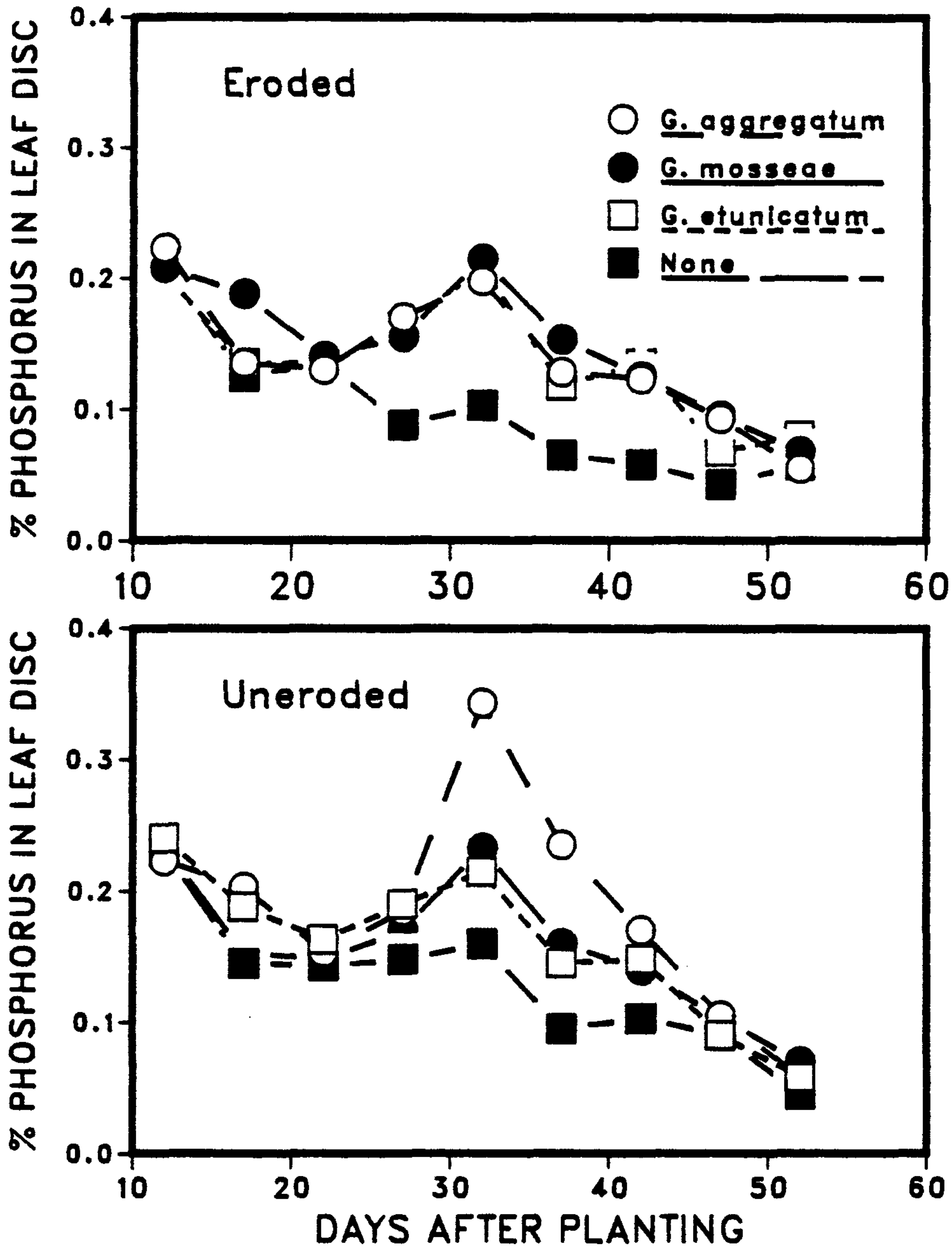

FIG. B.1. The influence of VAM inoculation on the development of mycorrhizal effectiveness in cowpea grown in eroded or uneroded soil.

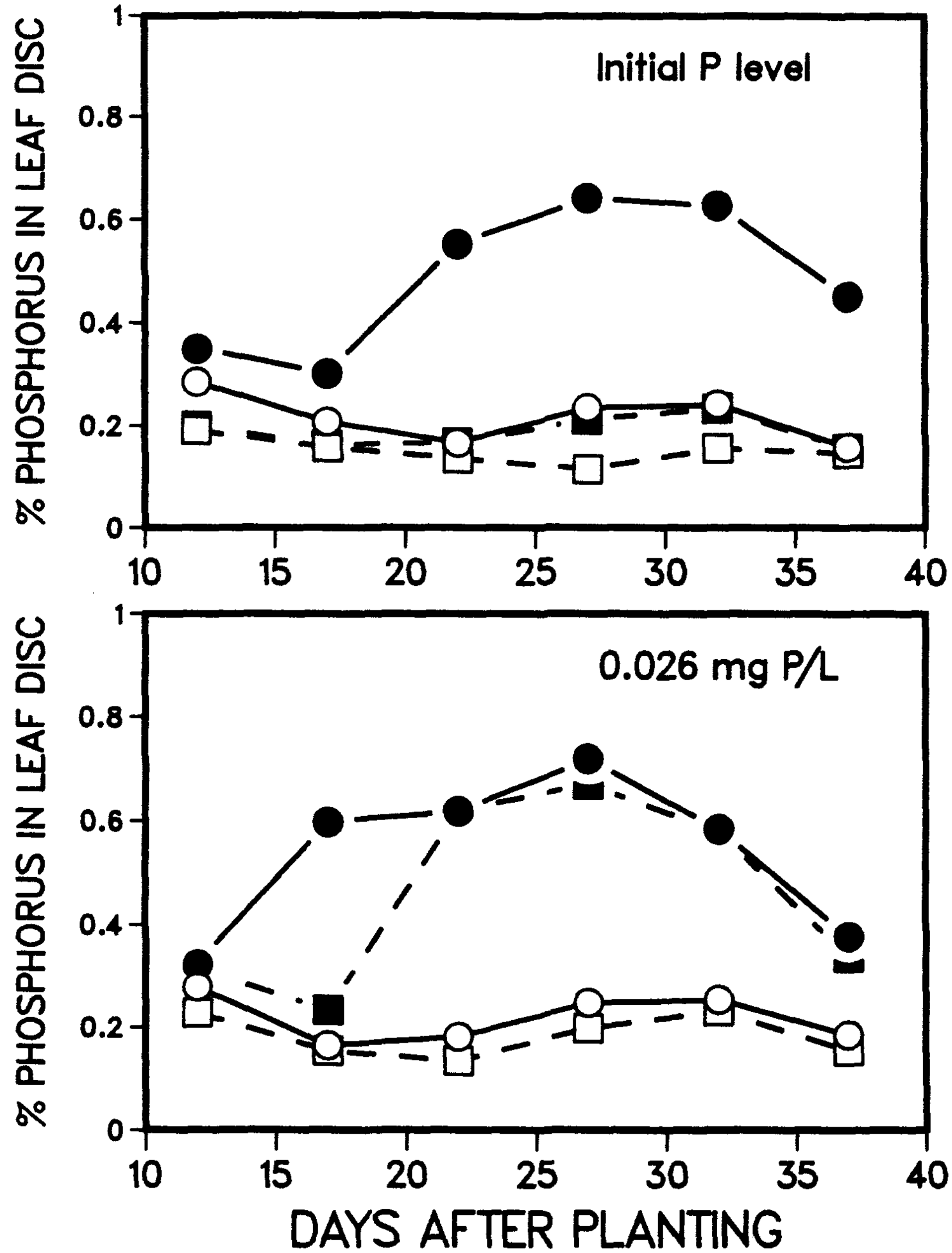

FIG. B.2. The influence of P and VAM inoculation on the development of mycorrhizal effectiveness in cowpea grown in eroded or uneroded soil. (○) = uneroded, uninoculated; (●) = uneroded, inoculated; (□) = eroded, uninoculated; (■) = eroded, inoculated.

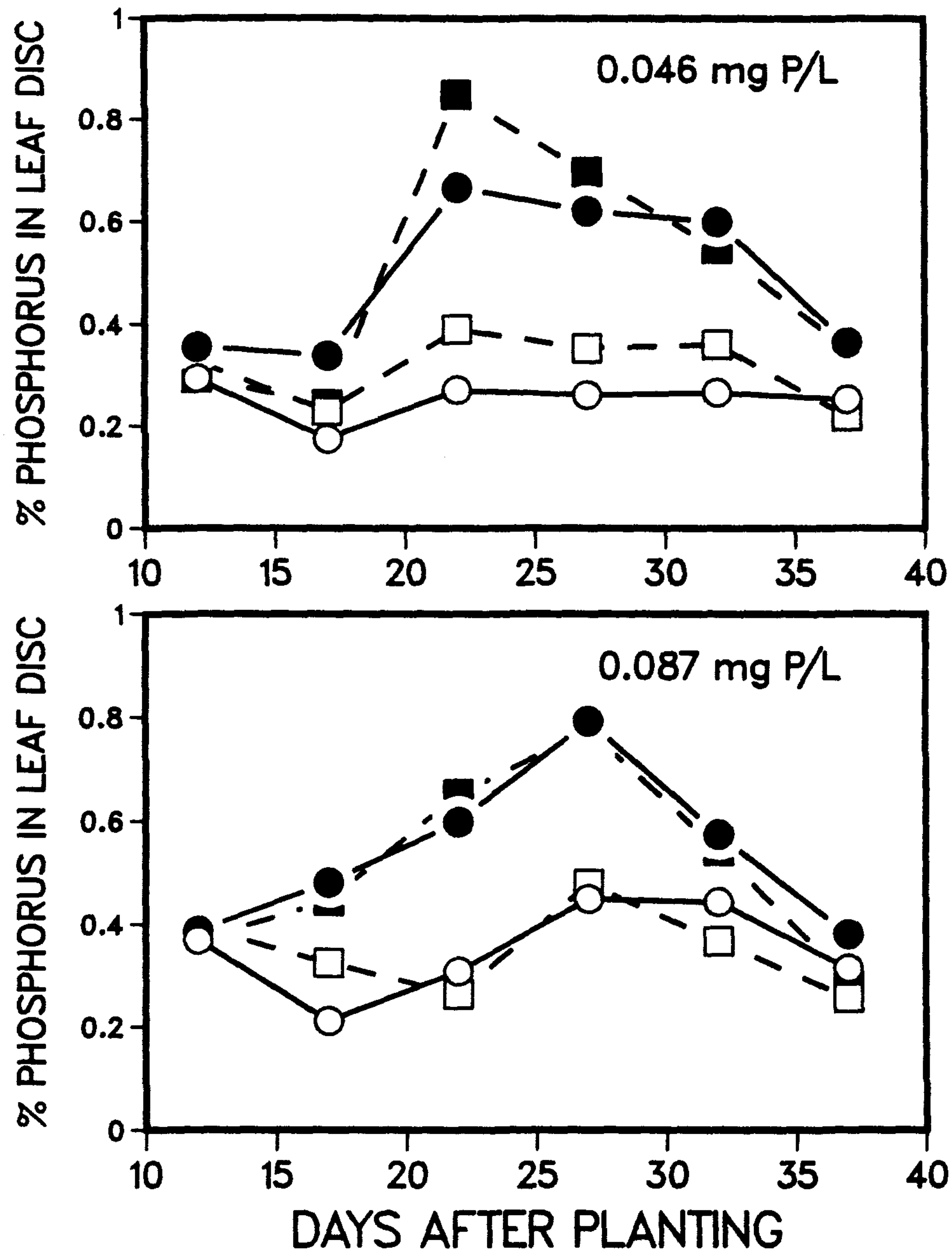

FIG. B.2. Continuation.

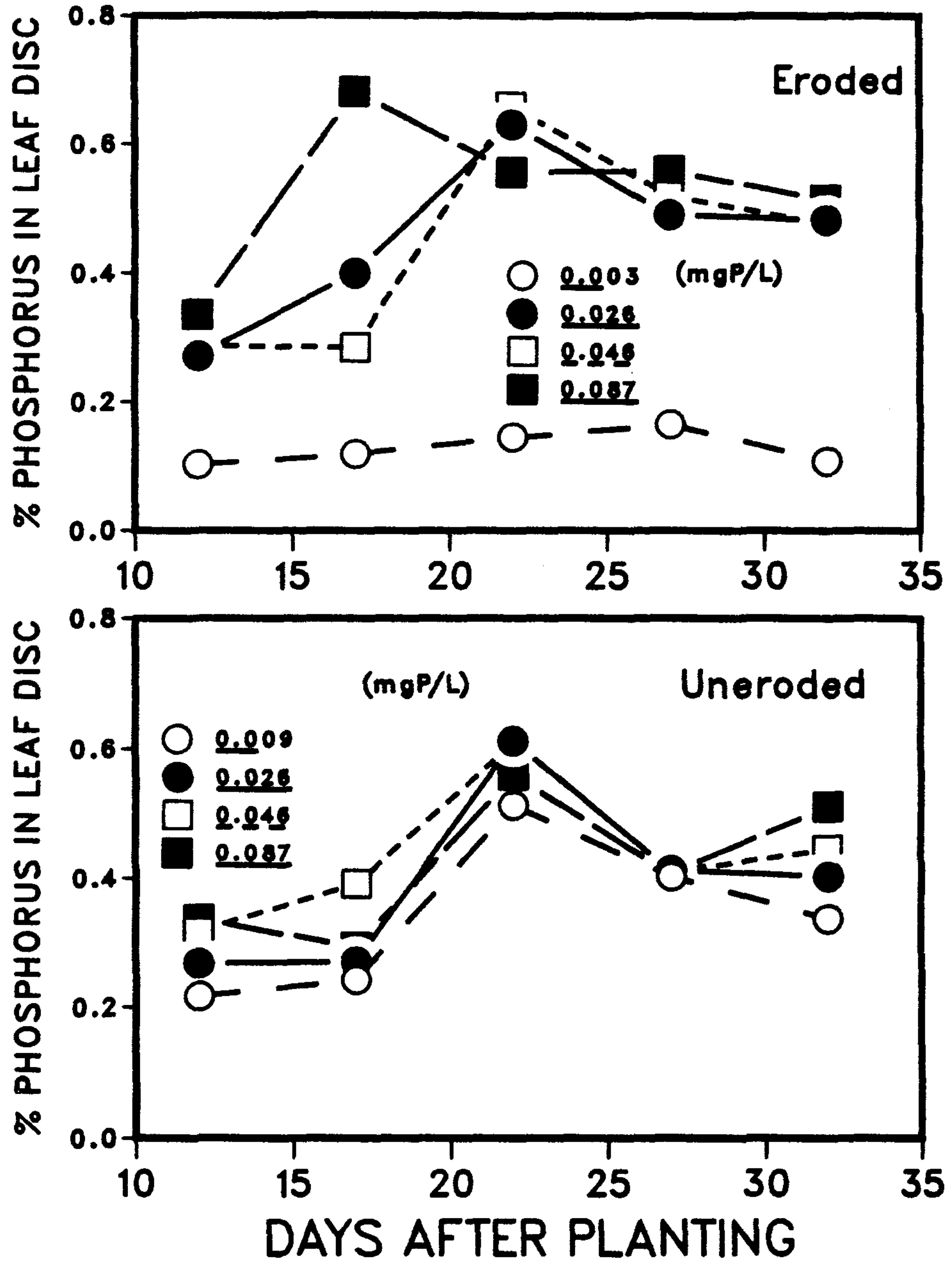

FIG. B.3. The influence of P on the development of mycorrhizal effectiveness in cowpea grown in eroded or uneroded soil inoculated with _G. aggregatum_.

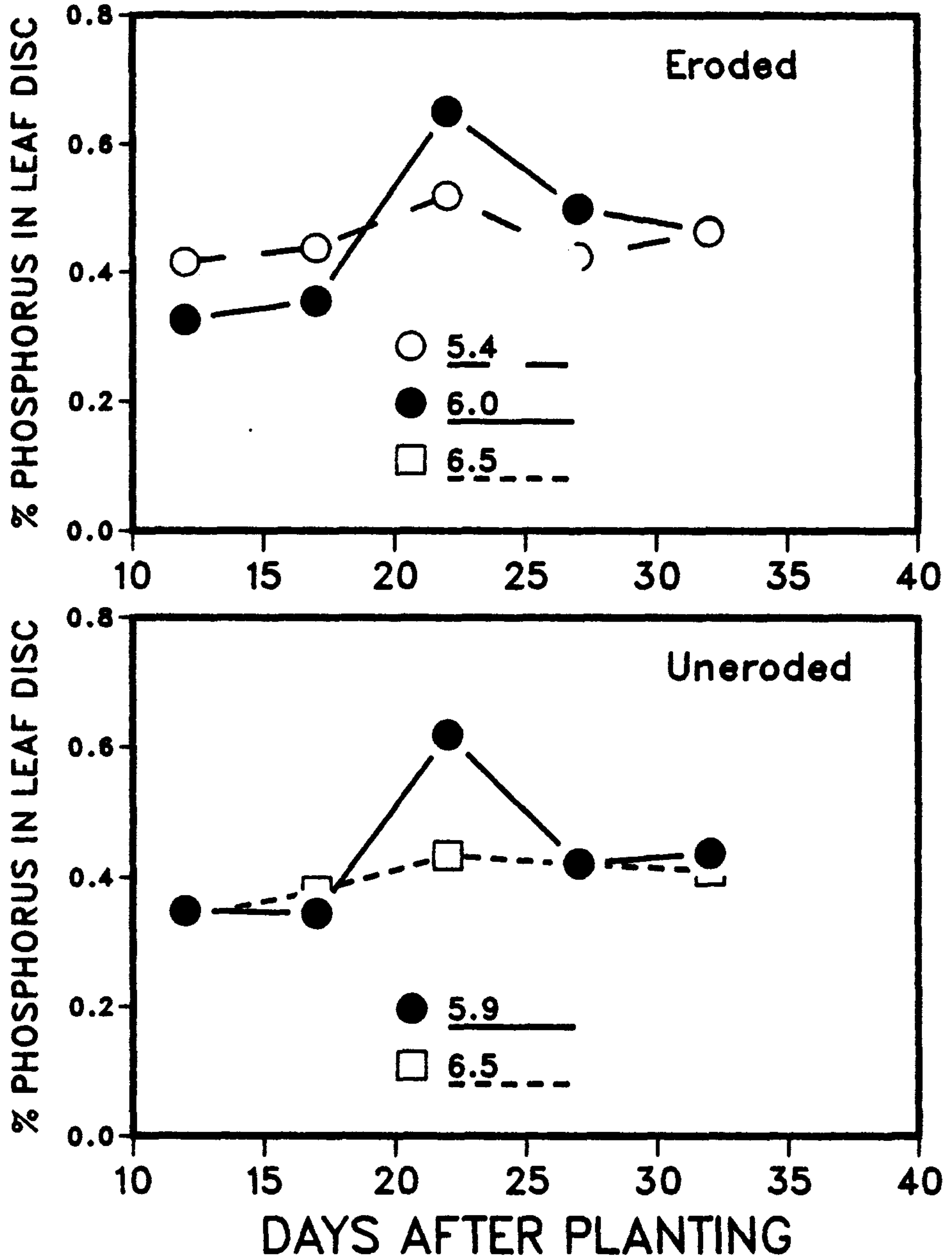

FIG. B.4. The influence of liming on the development of mycorrhizal effectiveness in cowpea grown in eroded or uneroded soil inoculated with G. aggregatum.

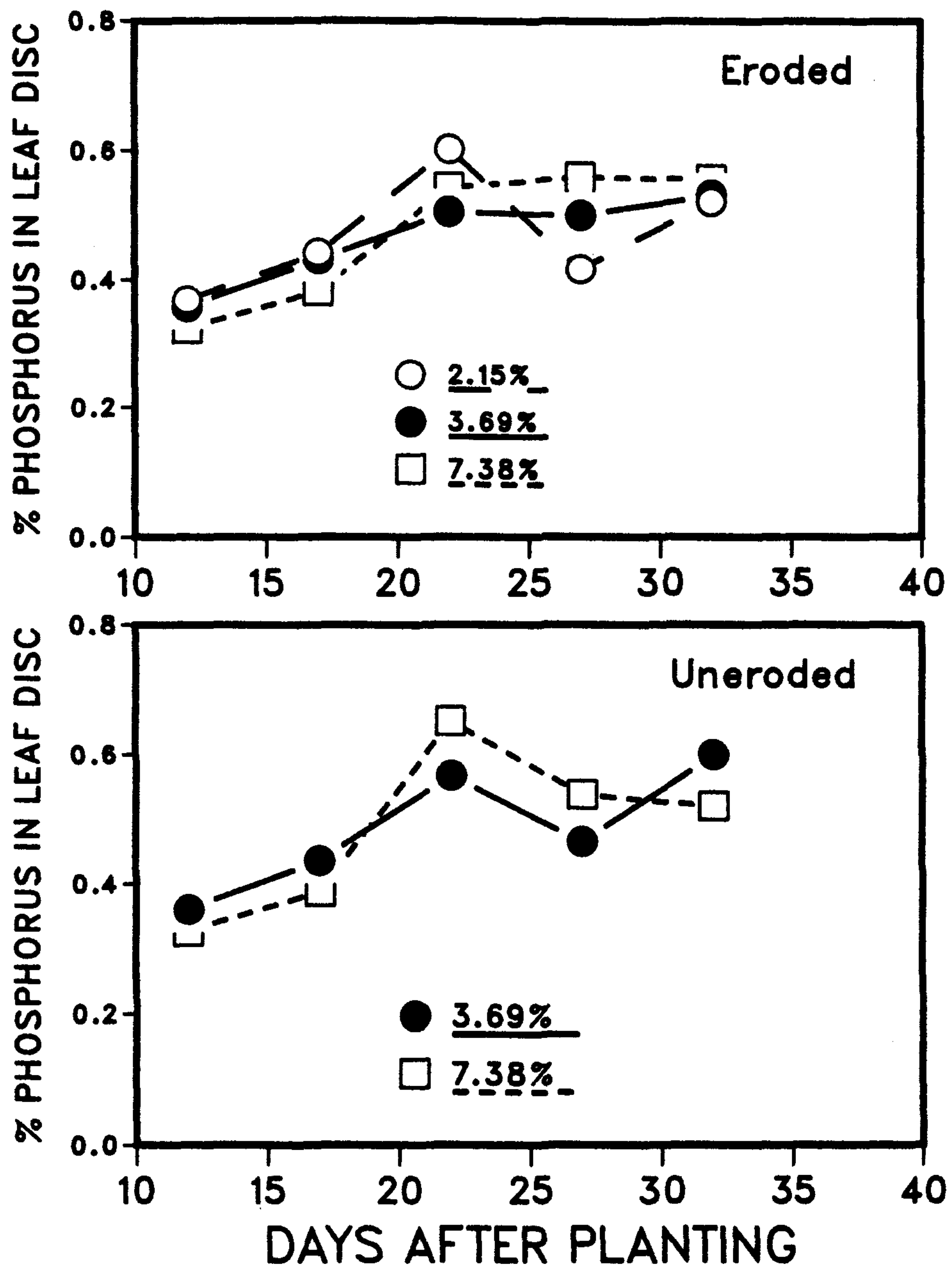

FIG. B.5. The influence of organic residue on the development of mycorrhizal effectiveness in cowpea grown in eroded or uneroded soil inoculated with G. aggregatum.

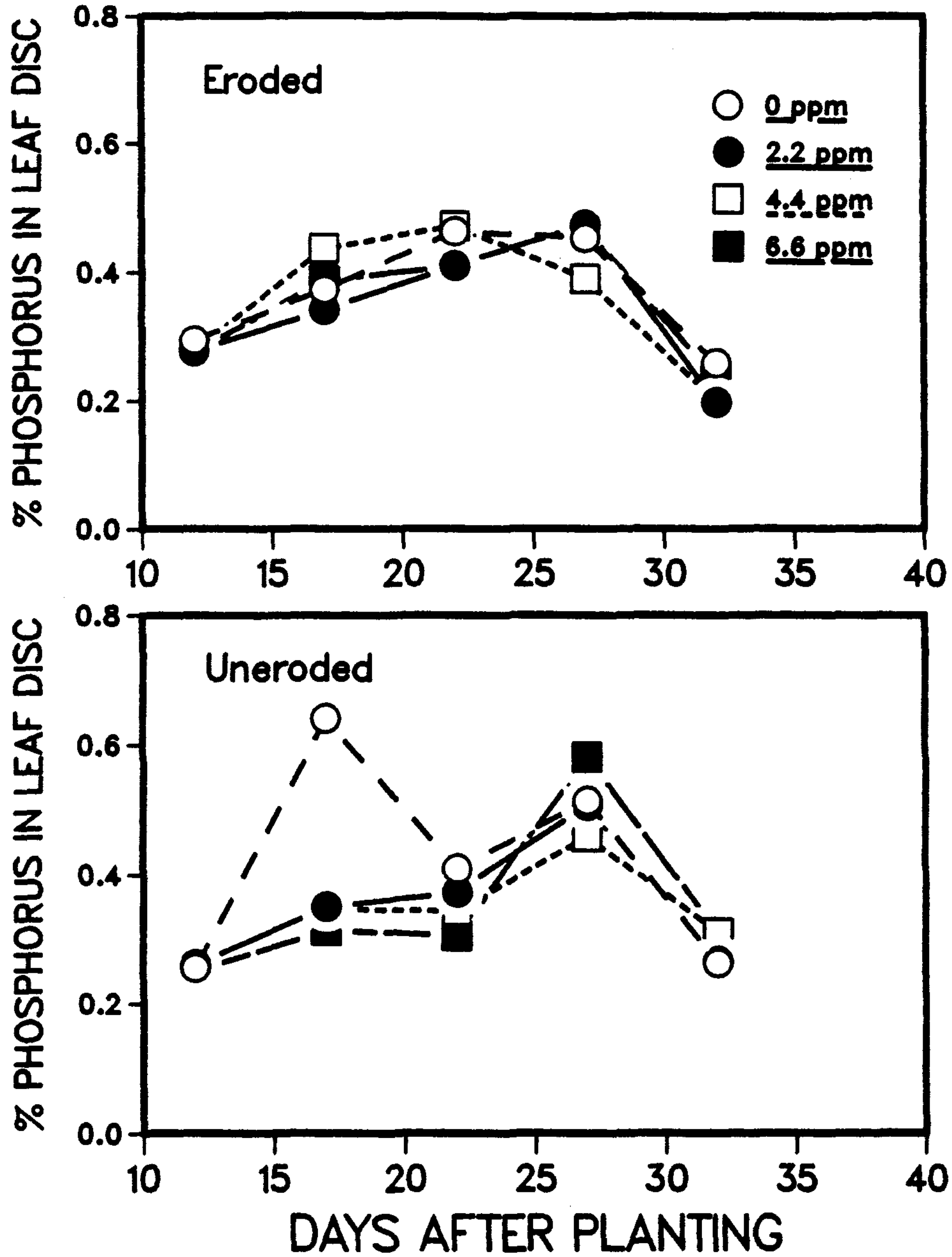

FIG. B.6. The influence of Mo on the development of mycorrhizal effectiveness in cowpea grown in eroded or uneroded soil inoculated with G. aggregatum.

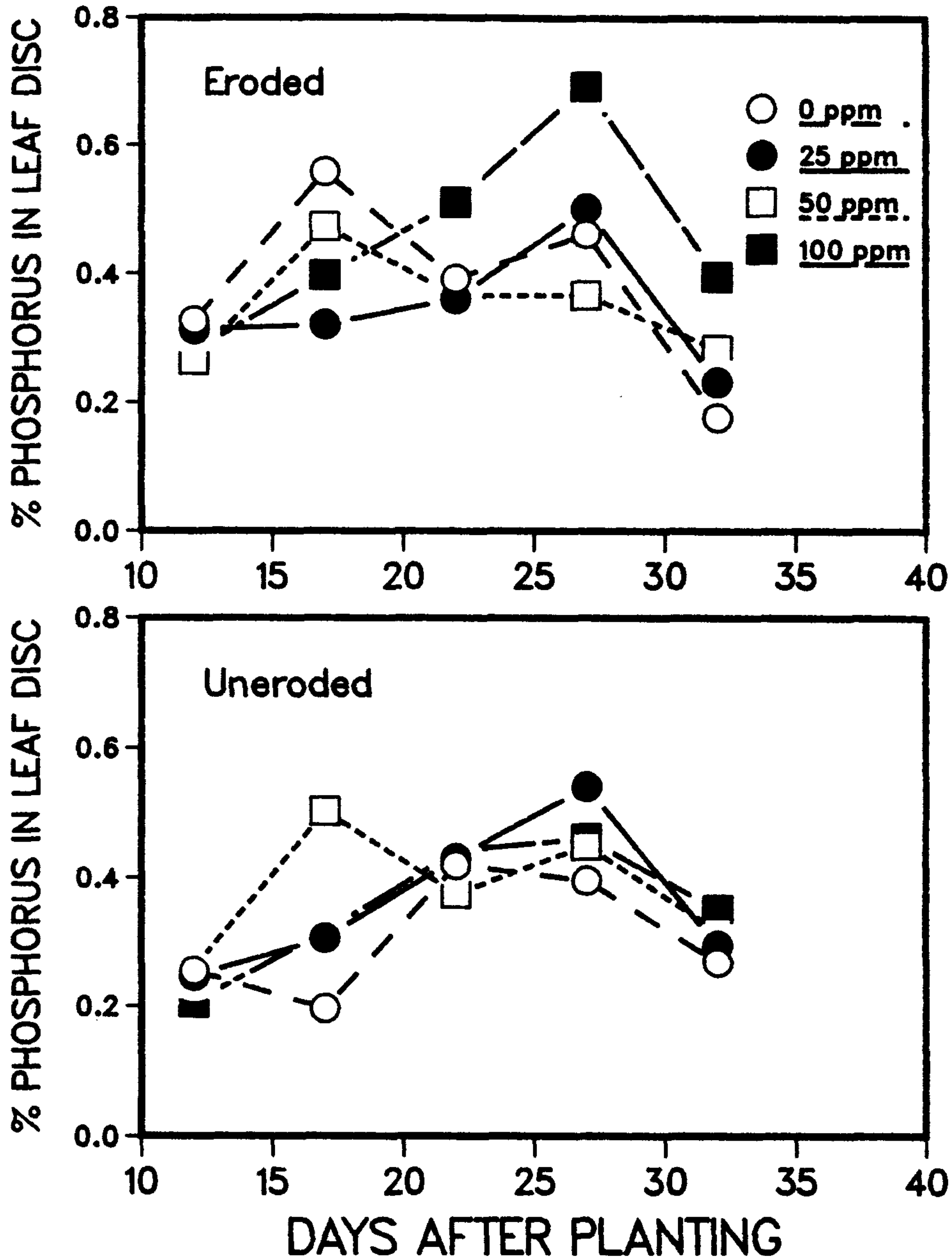

FIG. B.7. The influence of inorganic N on the development of mycorrhizal effectiveness in cowpea grown in eroded or uneroded soil inoculated with G. aggregatum.

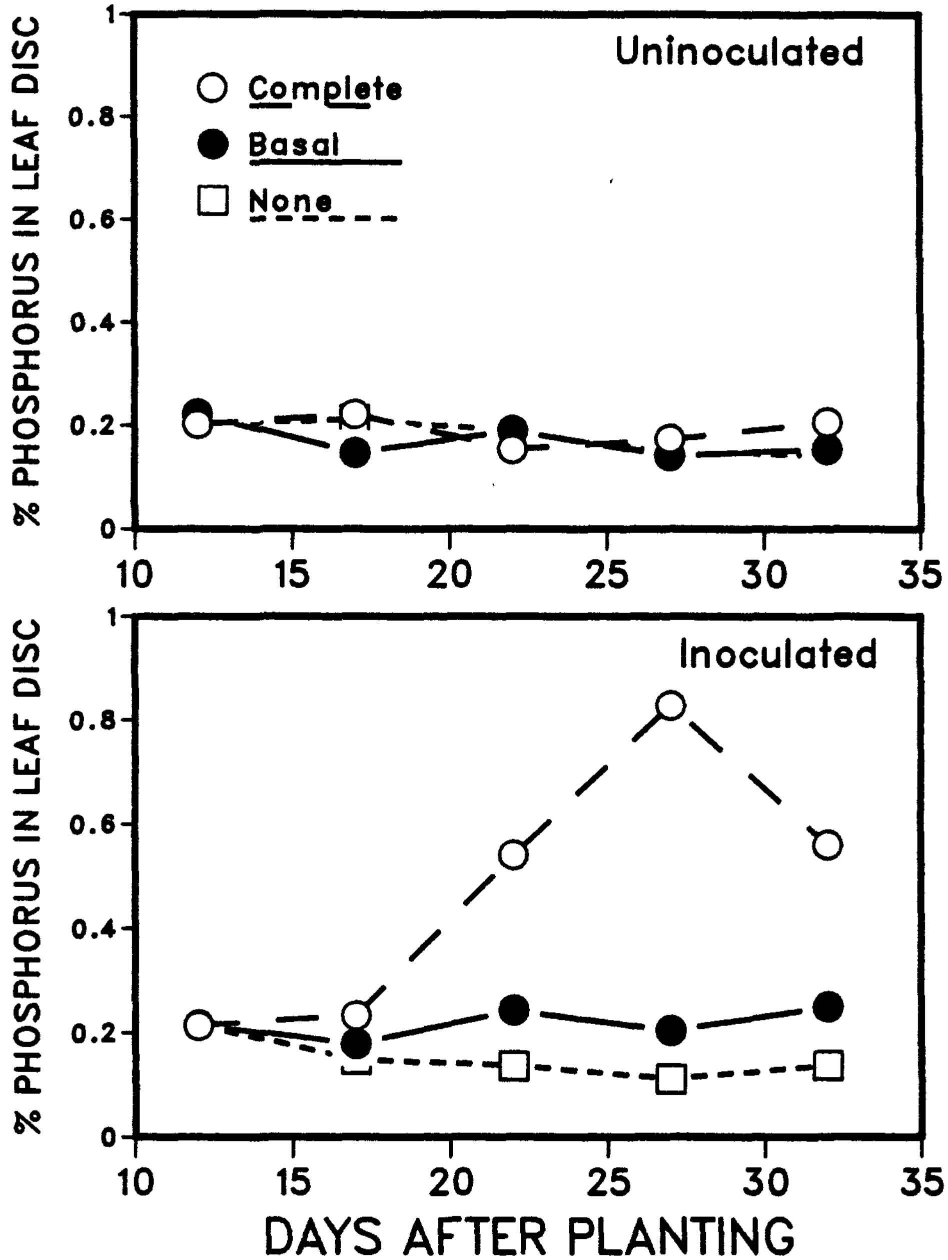

FIG. B.8. The influence of nutrient amendments and VAM inoculation on the development of mycorrhizal effectiveness in cowpea grown in eroded soil.

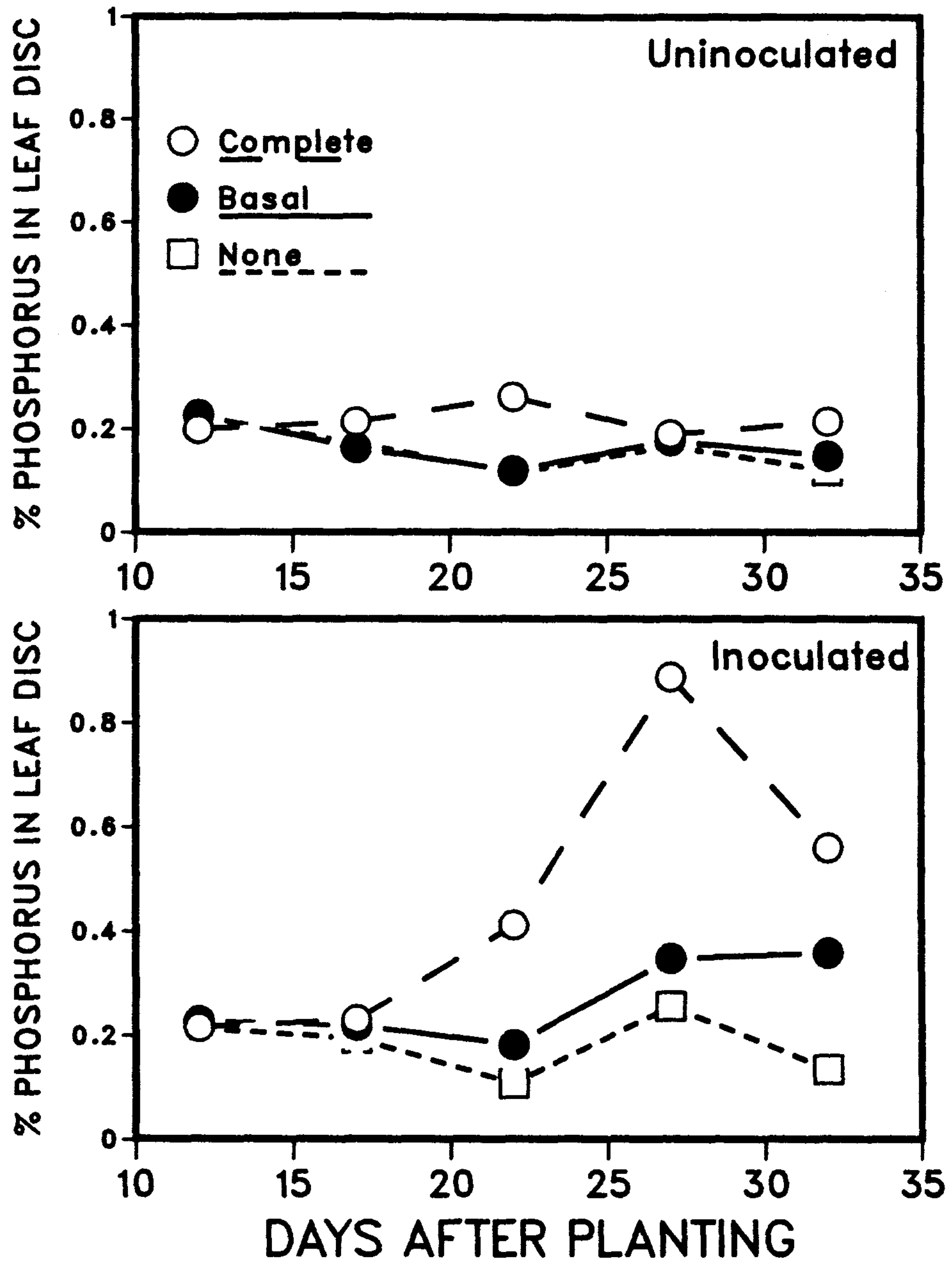

FIG. B.9. The influence of nutrient amendments and VAM inoculation on the development of mycorrhizal effectiveness in cowpea grown in uneroded soil.

APPENDIX C

CONCENTRATION OF P IN LEUCAENA SUBLEAFLETS (FIG. 1-8)

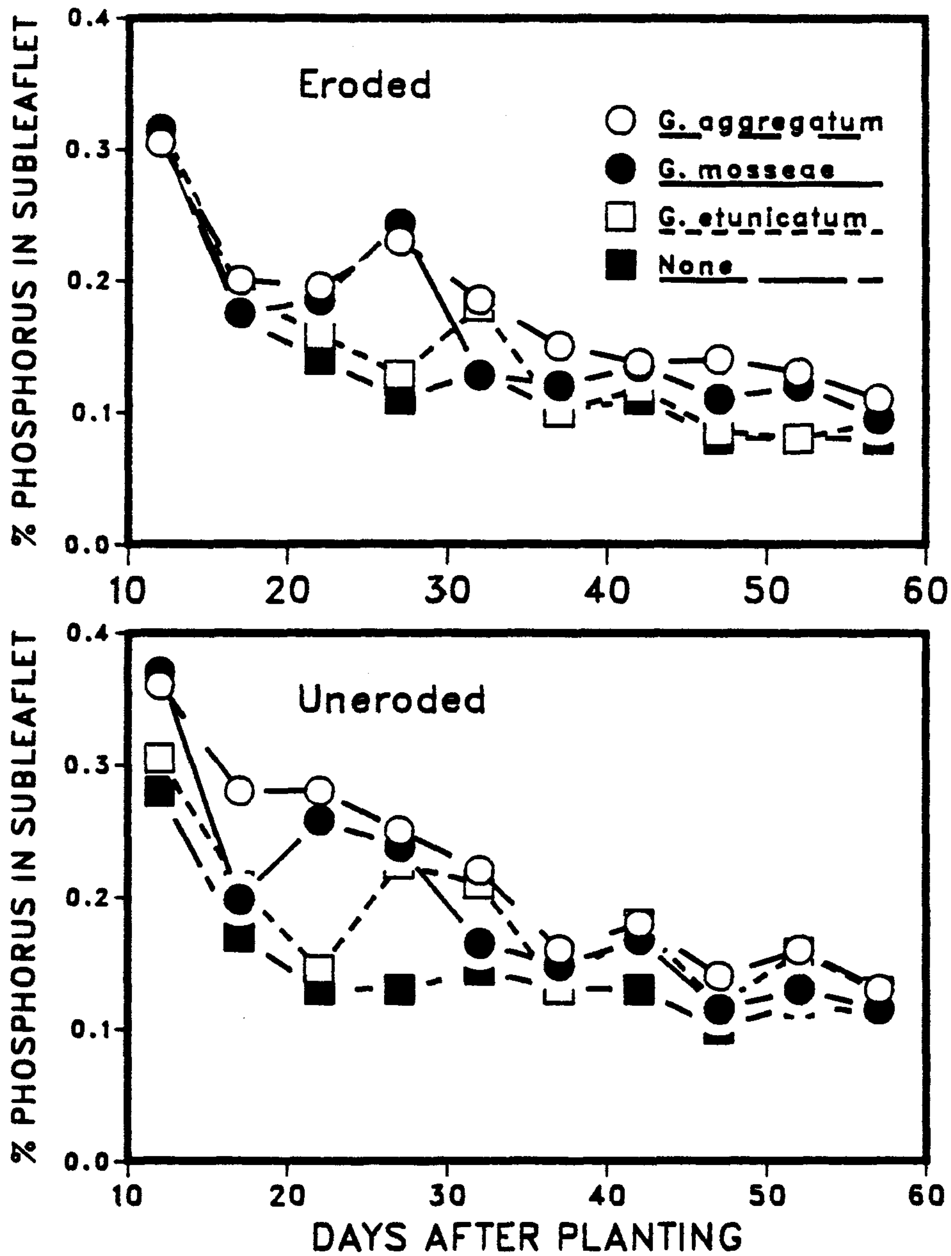

FIG. C.1. The influence of VAM inoculation on the development of mycorrhizal effectiveness in leucaena grown in eroded or uneroded soil.

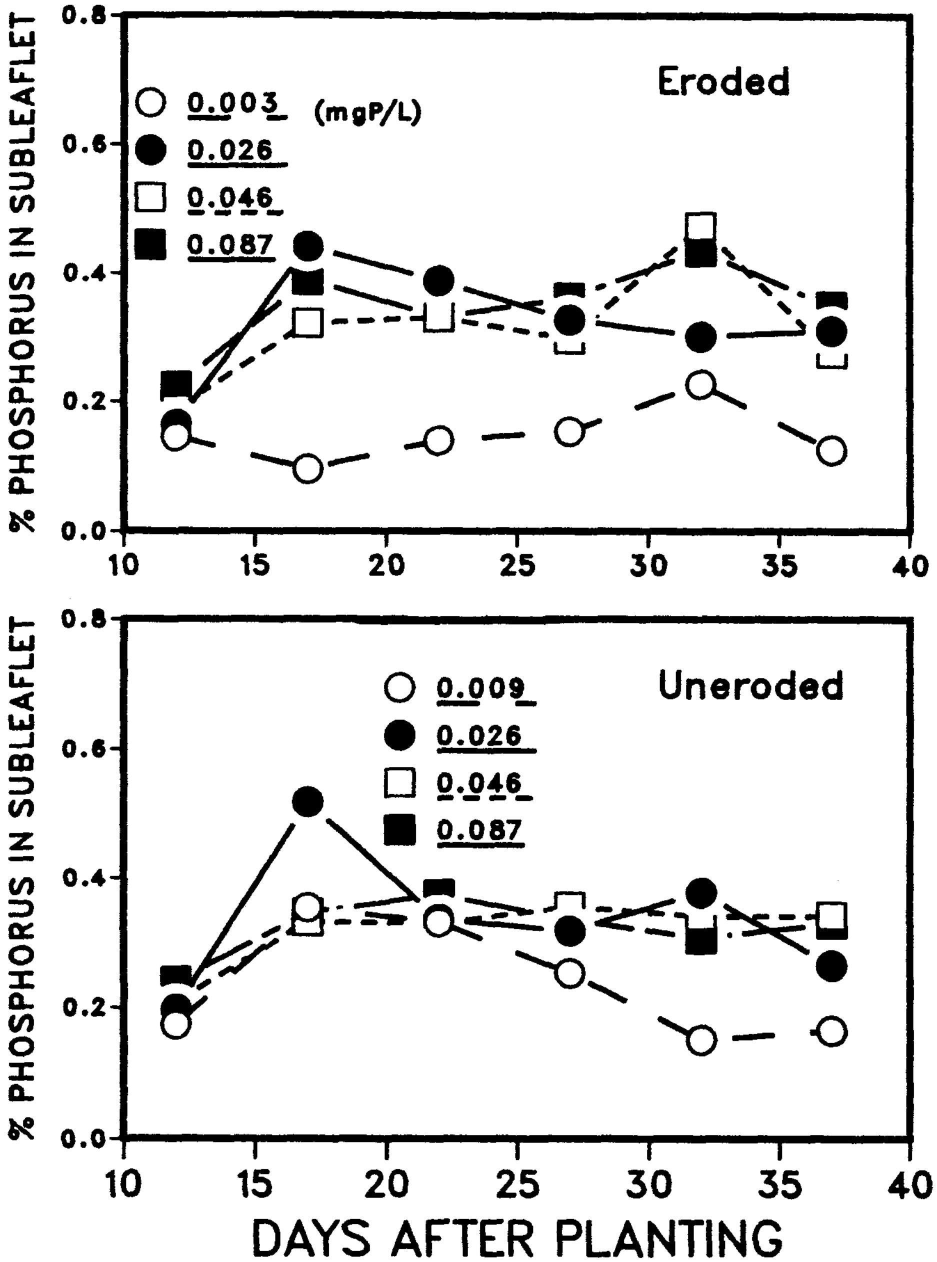

FIG. C.2. The influence of P on the development of mycorrhizal effectiveness in leucaena grown in eroded or uneroded soil inoculated with _G. aggregatum_.

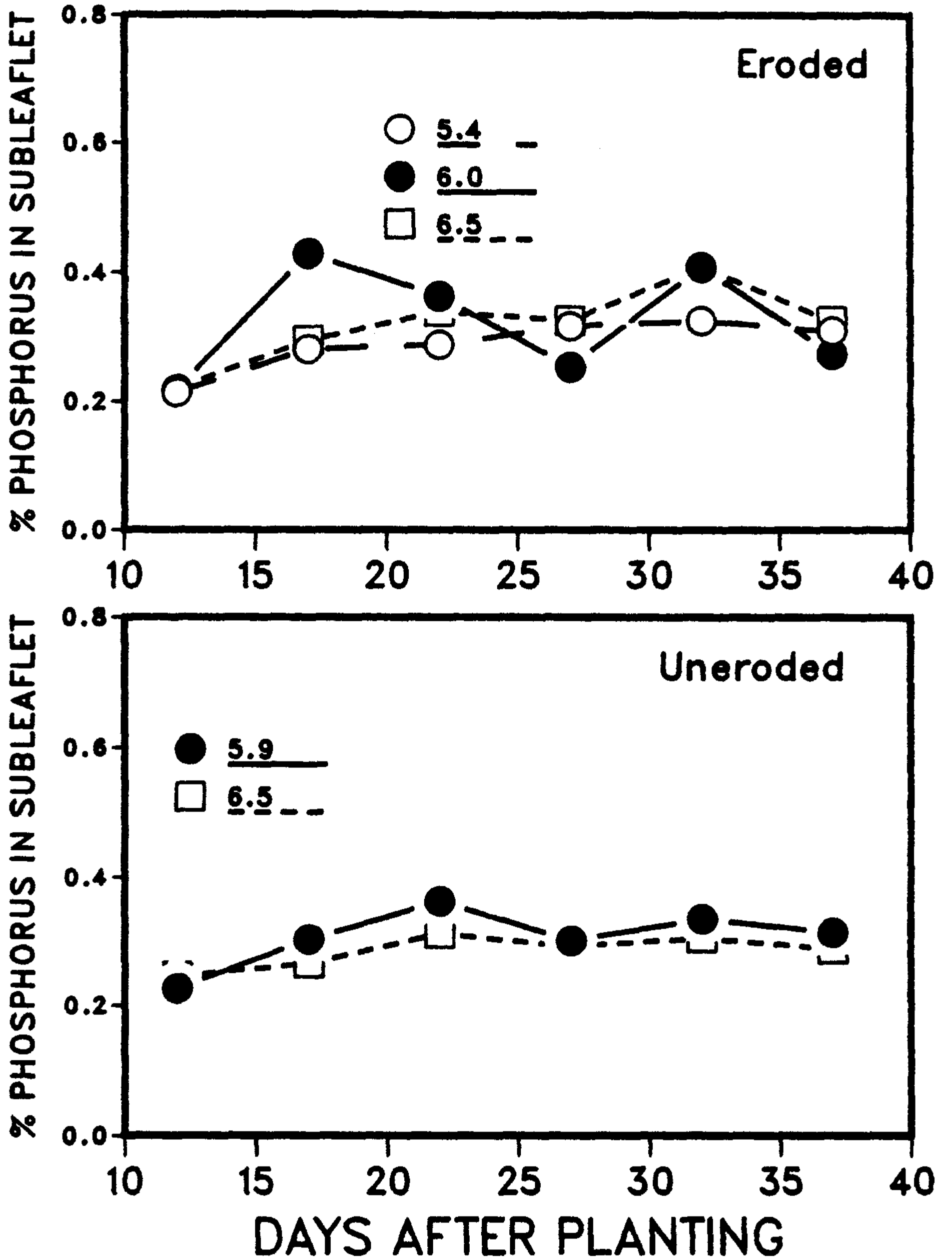

FIG. C.3. The influence of liming on the development of mycorrhizal effectiveness in leucaena grown in eroded or uneroded soil inoculated with G. aggregatum.

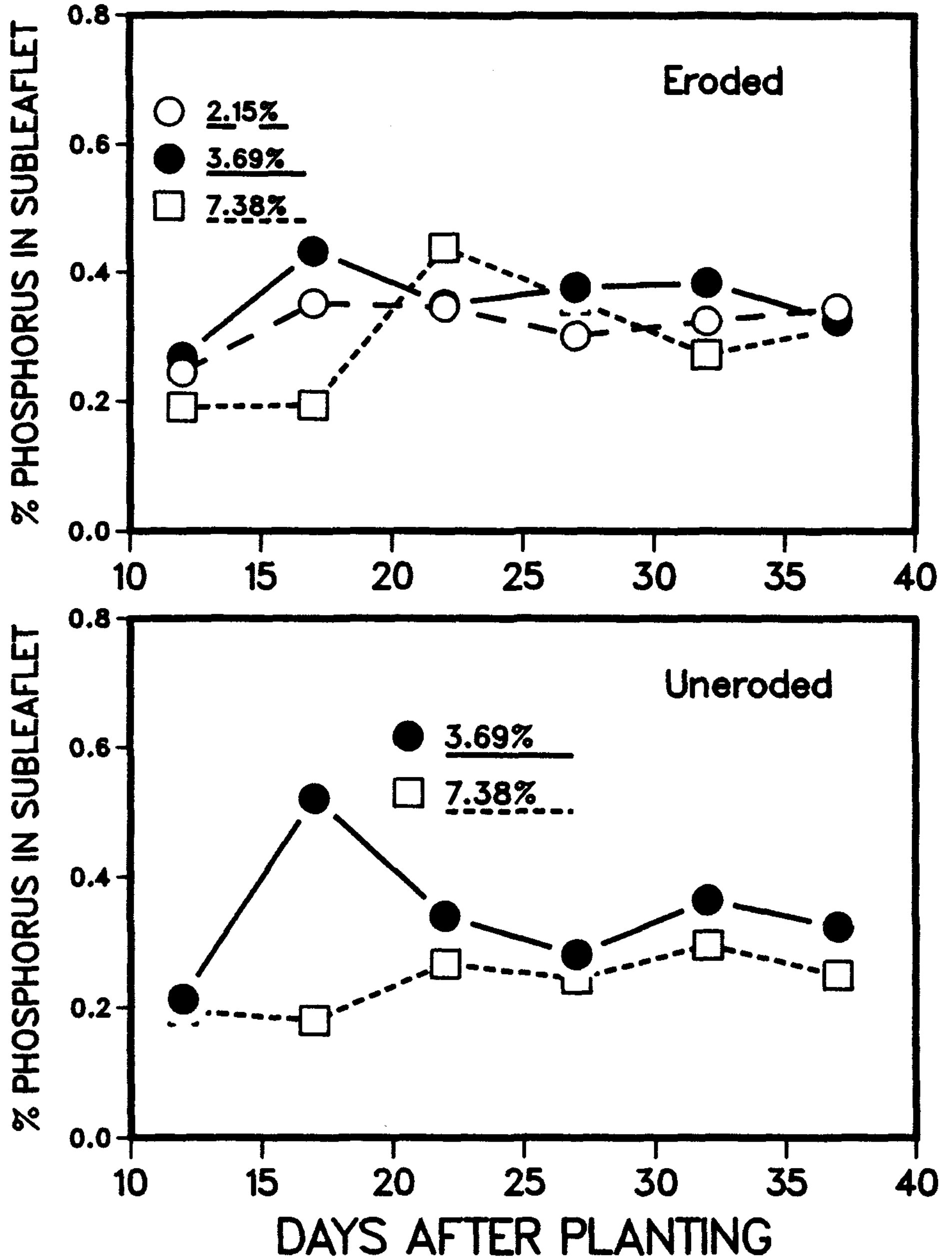

FIG. C.4. The influence of organic residue on the development of mycorrhizal effectiveness in leucaena grown in eroded or uneroded soil inoculated with G. aggregatum.

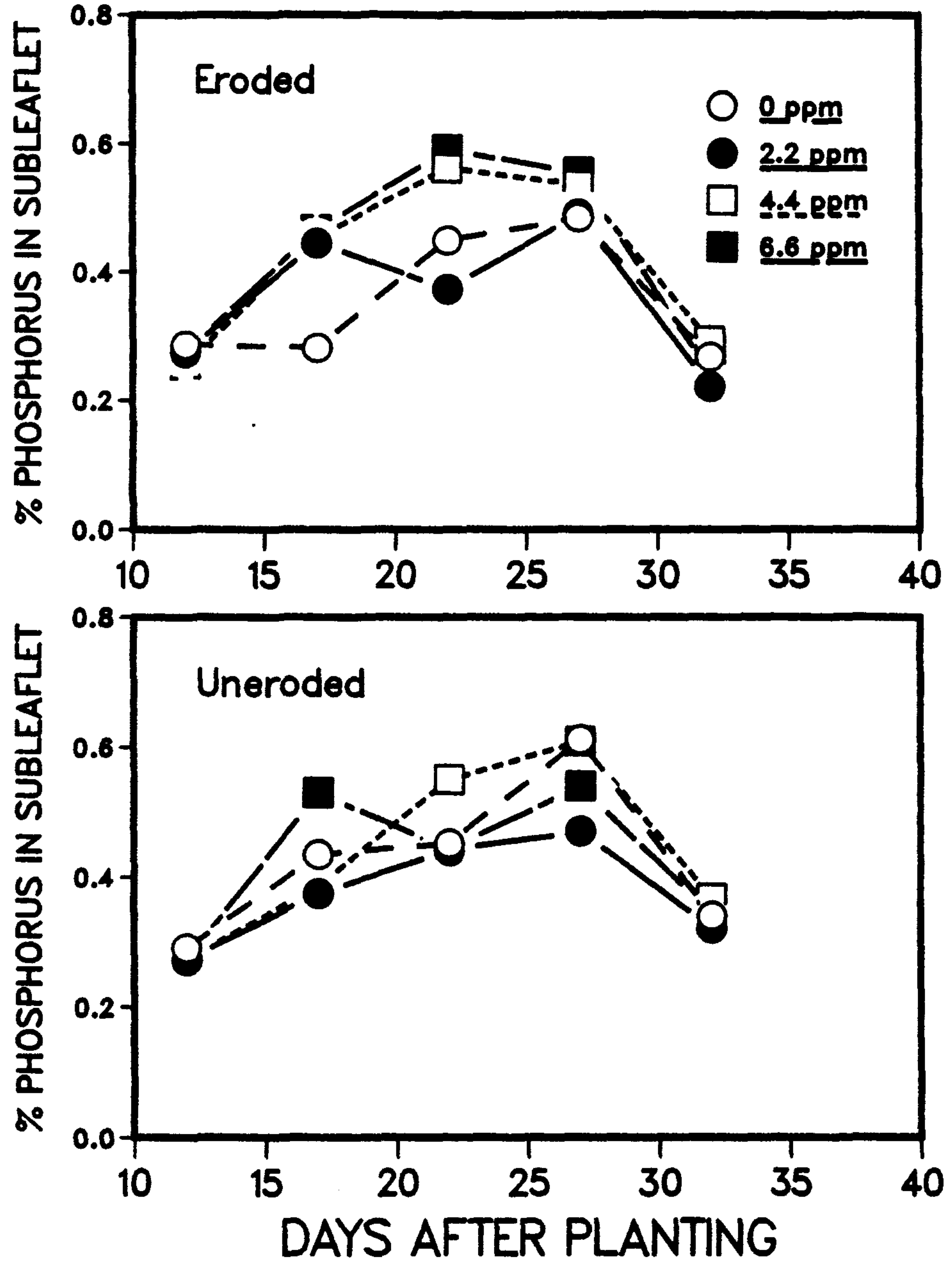

FIG. C.5. The influence of Mo on the development of mycorrhizal effectiveness in leucaena grown in eroded or uneroded soil inoculated with G. aggregatum.

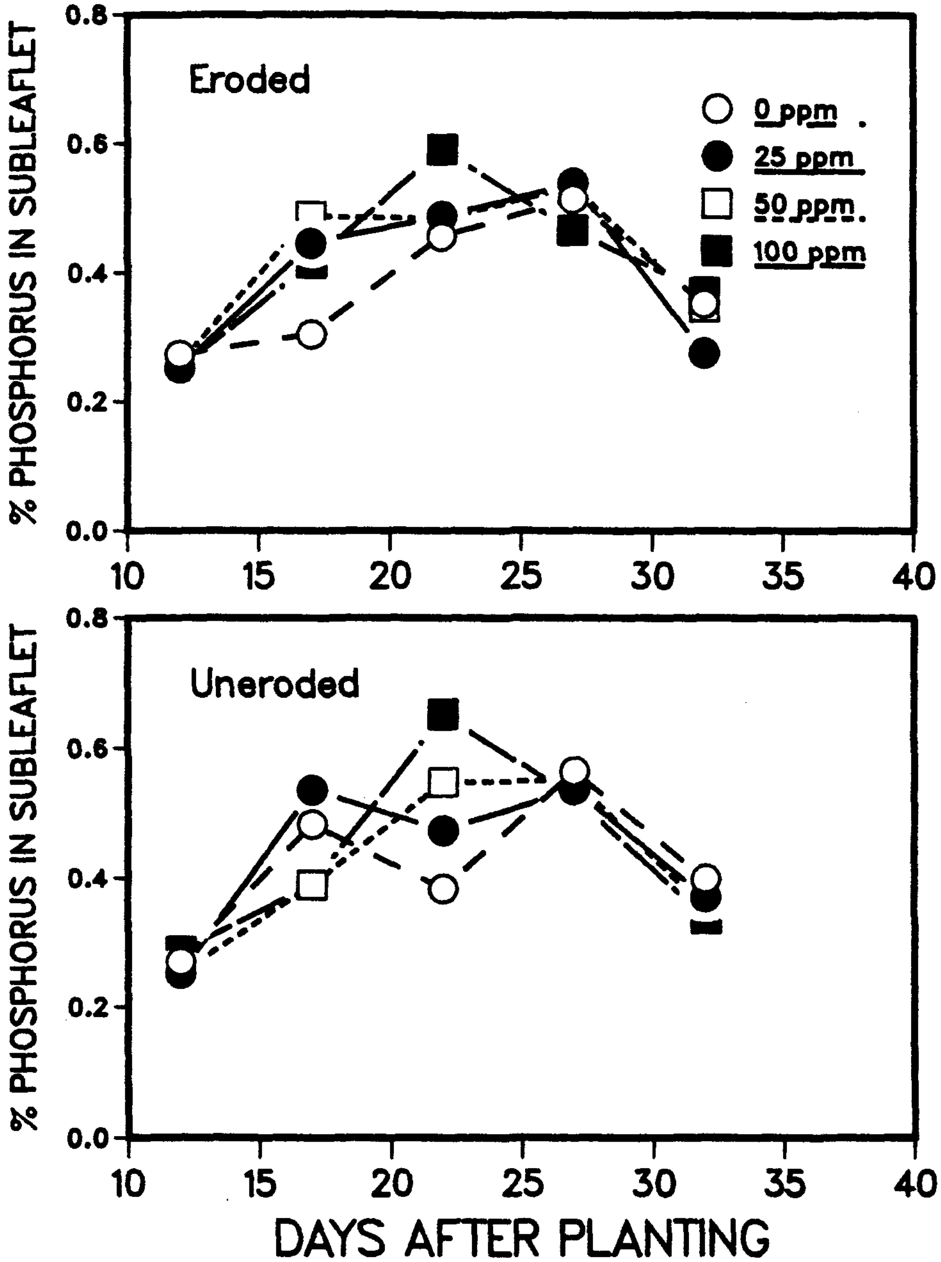

FIG. C.6. The influence of inorganic N on the development of mycorrhizal effectiveness in leucaena grown in eroded or uneroded soil inoculated with *G. aggregatum*.

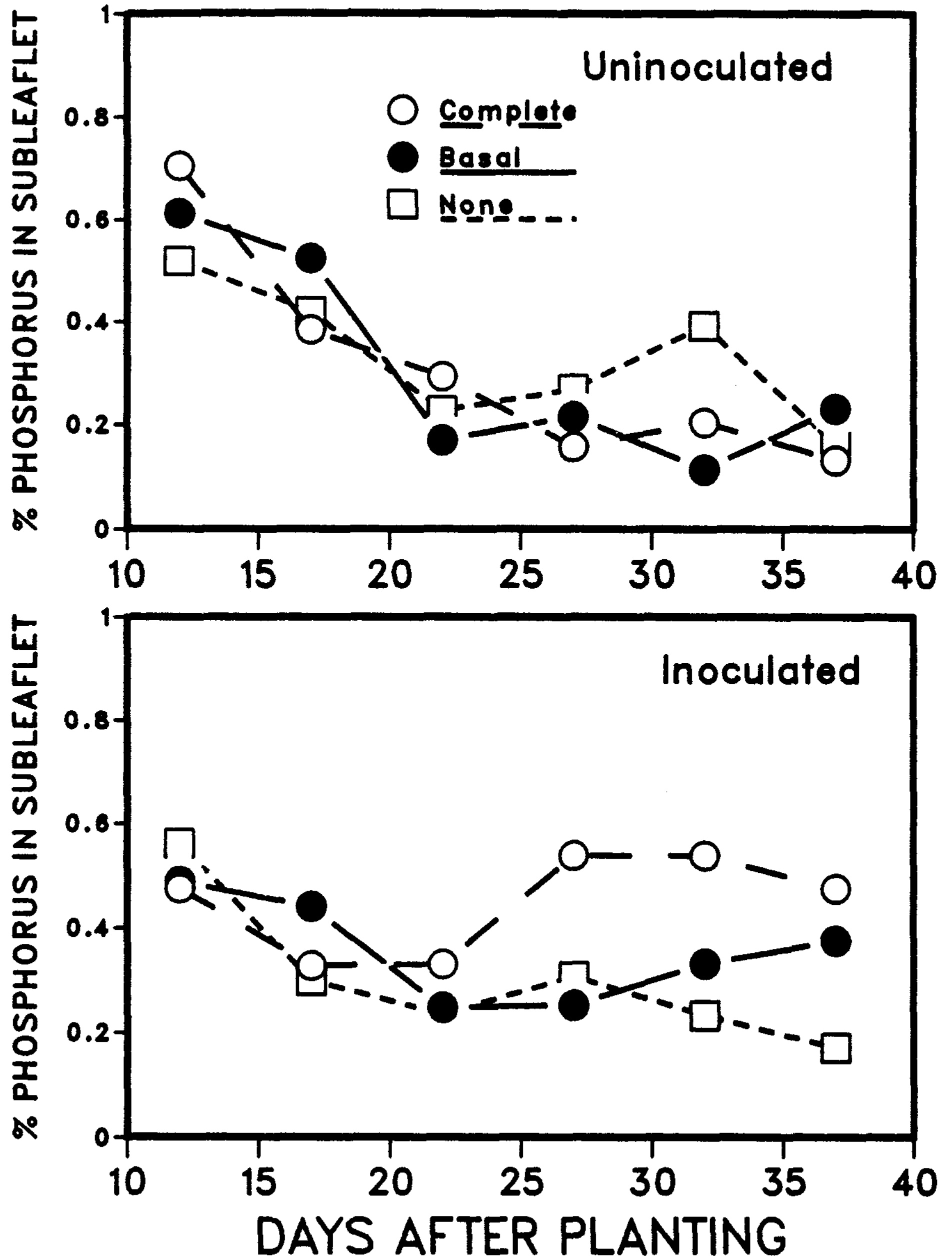

FIG. C.7. The influence of nutrient amendments and VAM inoculation on the development of mycorrhizal effectiveness in leucaena grown in eroded soil.

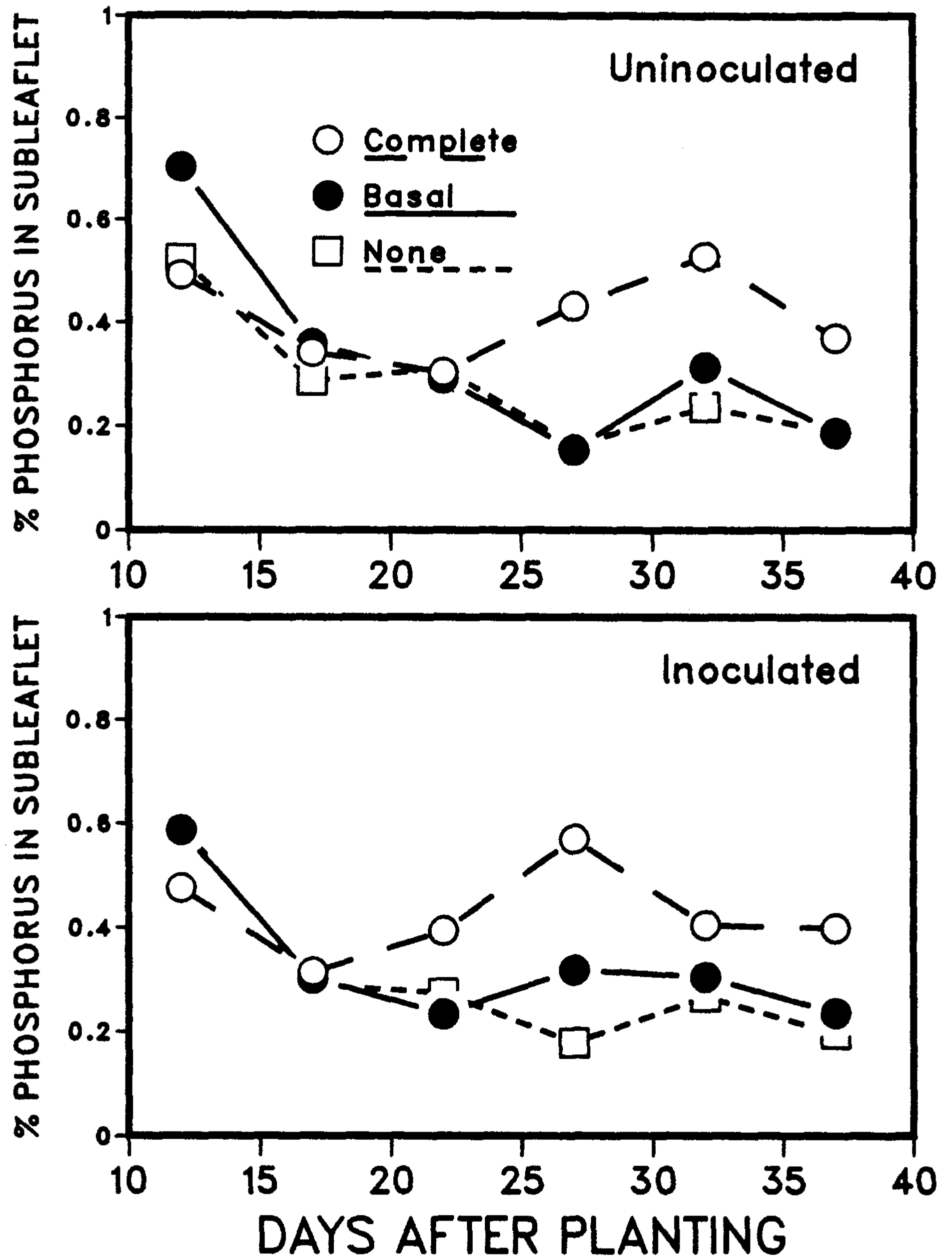

FIG. C.8. The influence of nutrient amendments and VAM inoculation on the development of mycorrhizal effectiveness in leucaena grown in uneroded soil.